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Hydroacoustic fish stock assessment in the presence of dense aggregations of *Chaoborus* larvae

Tommi Malinen, Antti Tuomaala, and Heikki Peltonen

Abstract: A new method for eliminating reverberation due to *Chaoborus* larvae from hydroacoustic recordings is presented based on an assumption of a constant dependence between the area backscattering strength (s_a) with a high-volume backscattering threshold (s_v) and s_a with a low s_v threshold for fish. The idea was to analyze data with a threshold high enough to eliminate reverberation and then convert the estimate to coincide with the result that would have been achieved with a low threshold containing all backscattering from fish. The approach was validated with a secondary dataset, and the magnitude of overestimation of fish density by reverberation was evaluated using data from four surveys conducted in a clay-turbid lake, where small planktivorous fish, smelt (*Osmerus eperlanus*), and larvae of *Chaoborus flavicans* coexist in the water column. With the presented method, estimation of smelt density was possible even when *Chaoborus* density was >200 individuals·m^{-3}. The analyses revealed that the overestimation of fish density could be as high as 50% if the reverberation is not taken into account. The presented method might also be applicable for eliminating reverberation due to other unwanted targets, because it is based on the acoustic properties of fish rather than those of unwanted targets.

Résumé : Notre nouvelle méthode pour éliminer la réverbération causée par les larves de *Chaoborus* dans les enregistrements hydroacoustiques se base sur la présupposition de l'existence d'une dépendance constante entre l'intensité de la rétrodiffusion par surface (s_a) à un seuil élevé de rétrodiffusion par volume (s_v), d'une part, et s_a à un seuil s_v bas pour les poissons, d'autre part. Le principe consiste à analyser les données à un seuil assez élevé pour éliminer la réverbération et de convertir ensuite l'estimation pour la faire coïncider avec le résultat qui aurait été obtenu avec un seuil bas qui contient toute la rétrodiffusion due aux poissons. L'utilisation d'une série secondaire de données a servi à valider la méthodologie; des données de quatre inventaires réalisés dans un lac turbide à l'argile où cohabitent dans la colonne d'eau des éperlans (*Osmerus eperlanus*), des petits poissons planctonophages, et des larves de *Chaoborus flavicans* ont permis d'évaluer l'importance de la surestimation de la densité de poissons à cause de la réverbération. Avec notre méthode, il est possible d'estimer la densité des éperlans même quand la densité des *Chaoborus* est >200 individuels·m^{-3}. Les analyses révèlent que la surestimation peut atteindre 50 % lorsqu'on néglige la réverbération. Notre méthode peut probablement aussi servir à éliminer la réverbération causée par d'autres cibles non désirées, parce qu'elle se base sur les propriétés acoustiques des poissons plutôt que sur celles des cibles accessoires.

[Traduit par la Rédaction]

Introduction

Hydroacoustics is a method that enables quantitative estimates of fish abundance and spatial distribution. Although the estimates are accurate in optimal conditions, the method is subject to many sources of bias. One of them is reverberation, i.e., echoes from unwanted targets (MacLennan and Simmonds 1992). In particular, aggregations of *Chaoborus* larvae can produce echoes as strong as those from small fishes (Eckmann 1998).

Typically, most *Chaoborus* inhabit the lake sediment in the daytime but ascend to epilimnion at night, inducing an overlap of fish and *Chaoborus* distributions (Teraguchi and Northcote 1966). This noticeably reduces the applicability of

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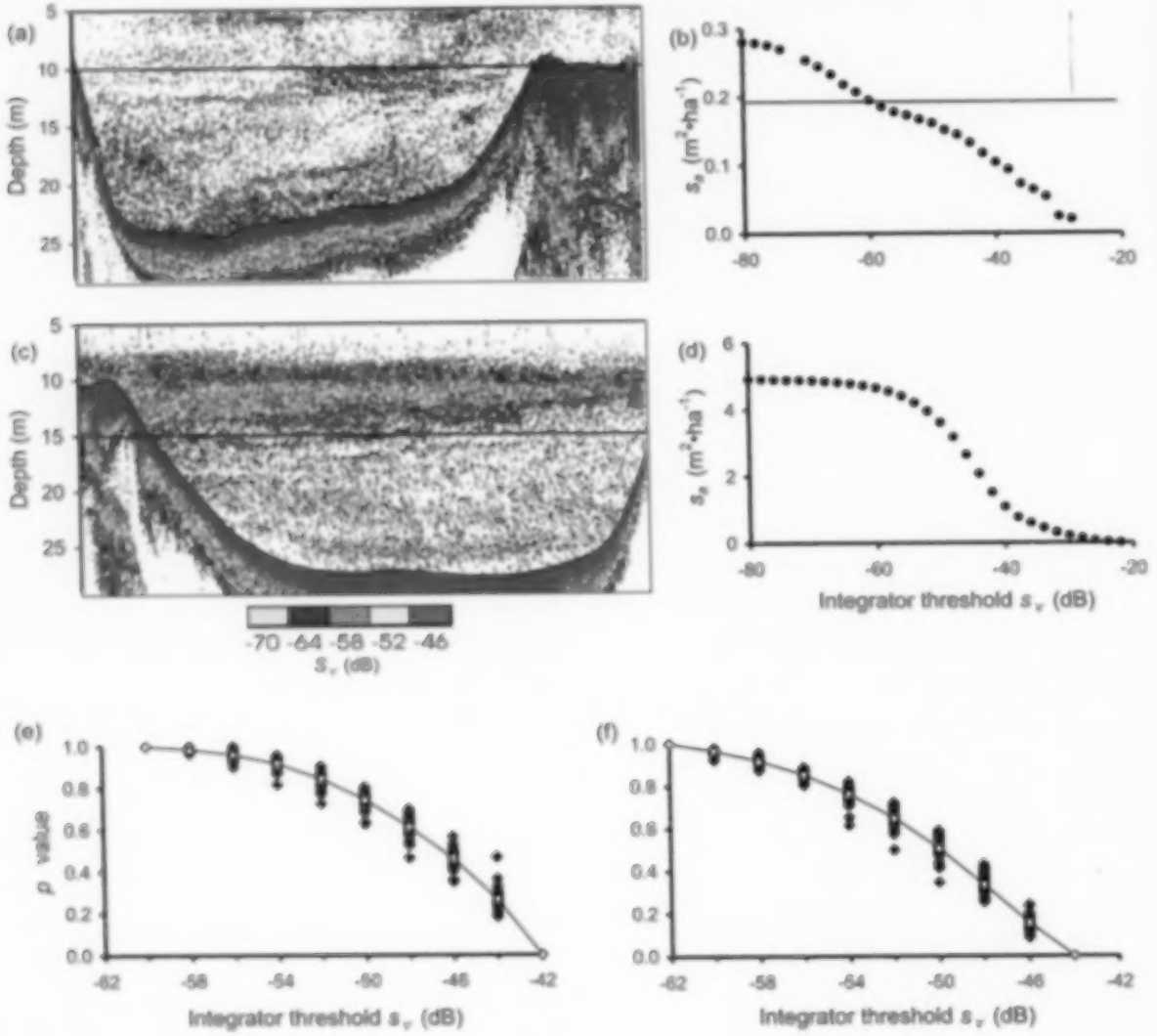
Fig. 1. (*a* and *b*) An echogram and s_a as a function of s_v threshold from low *Chaoborus* density where a plateau exists at an intermediate threshold level making Eckmann's method applicable (on 3 June 1999) and (*c* and *d*) from high density requiring a more efficient method (on 3 August 1999). (*e* and *f*) The relationship between s_v threshold and *p* value, i.e., relative s_a(f) in the range of our new analysis in (*e*) shallow and (*f*) deep layers. The horizontal line in (*b*) indicates an asymptote for the curve of s_a(f) determined by the Bertalanffy function. The horizontal lines in (*a*) and (*c*) represent the lower boundaries of analyzed water layers.



hydroacoustics for fish stock assessment, because without the disturbance by *Chaoborus*, nighttime, in particular, might be an optimal period for surveys (Appenzeller and Leggett 1992). The most problematic situation prevails in clay-turbid lakes, where larvae inhabit the water column also during daytime (Liljendahl-Nurminen et al. 2002). The thresholding method (Eckmann 1998) is applicable to discrimination between *Chaoborus* and fish in certain circumstances (Malinen et al. 2001). In this method, each acoustic dataset is analysed with several volume backscattering (s_v) thresholds, and curves of area backscattering strength (s_a) as a function of s_v threshold are constructed. If the curve shows some intermediate plateau before reaching the final plateau, the s_a from fish can be calculated, for instance, with an asymptotic Bertalanffy function. However, if dense aggregations of *Chaoborus* exist in water layers containing small fish, no intermediate plateau can be found (Fig. 1). In such cases, a more efficient method for elimination of the reverberation due to *Chaoborus* would greatly improve the accuracy of fish stock assessment. Although the problem has been frequently reported (Unger and Brandt 1989; Eckmann 1998; Horppila et al. 2000), no quantitative evaluation of bias on stock estimates caused by *Chaoborus* has been published.

In this paper, we present a new method for discriminating between *Chaoborus* larvae and fish and validate it with a

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secondary dataset. In addition, we estimate the bias caused by *Chaoborus* on fish stock estimates if the reverberation is not taken into account by using surveys made in a clay-turbid lake where small smelt (*Osmerus eperlanus*) and larvae of *Chaoborus flavicans* coexist in the water column (Horppila et al. 2004).

Material and methods

Field data

Hydroacoustic data for the current study were taken from 26 surveys conducted during 1999–2001, originally obtained for smelt stock assessment in eutrophic and clay-turbid Lake Hiidenvesi in southern Finland (Horppila et al. 2003). The data contained both day and night surveys conducted along 7–17 equidistant transects at intervals of 500–1400 m. The equipment used was a SIMRAD EY-500 echosounder (Simrad AS, N-3191 Horten, Norway) equipped with a 120 kHz split-beam transducer, and the data were analyzed with EP 500 software (Lindem Data Acquisition, Simrad AS, Horten, Norway). Additionally, simultaneous fishing with a small pelagic pair trawl was conducted over 11 surveys to determine fish species and size composition. The equipment and sampling procedure are described in Horppila et al. (2003).

Elimination of *Chaoborus* reverberation

To eliminate *Chaoborus* reverberation, we determined first the relationship between s_a and s_v thresholds using 88 samples (49 day and 39 night observations) containing only fish. We analyzed deep (≥14 m) and shallow (<14 m) water layers separately (Fig. 1), because we assumed that the relationship may change with depth, e.g., because of increased volume of analyzed cell in thresholding. Then we applied these relationships to analyzing fish densities by assuming a constant dependence between s_a with high threshold and s_a with low threshold for fish. The idea was to analyze the data with a threshold high enough to eliminate reverberation and then convert the estimate to coincide with the result that would have been achieved with a low threshold containing all backscattering from fish.

More exactly, the following formulation was applied for the estimation of area backscattering strength of fish, $s_a(f)$, in either a deep or a shallow layer of a transect:

$$(1) \qquad s_a(f) = \frac{s_a[\mathrm{thr(a)}] - s_a[\mathrm{thr(h)}]}{p[\mathrm{thr(a)}]} + s_a[\mathrm{thr(h)}]$$

where thr(a) is the s_v threshold applied, i.e., the lowest threshold eliminating *Chaoborus* reverberation. This was determined visually by thresholding for each sample. Several thresholds were tested in ascending order, and the first one eliminating all reverberation (i.e., also the strongest echoes from *Chaoborus*) was chosen. thr(h) is the upper limit of the analysis (–42 dB in shallow layers and –44 dB in deep layers). Above this limit, the p value, $p(\mathrm{thr}(a))$, was subject to unacceptably large variation.

$$(2) \qquad p[\mathrm{thr(a)}] = \frac{s_a[\mathrm{thr(a)}] - s_a[\mathrm{thr(h)}]}{s_a[\mathrm{thr(l)}] - s_a[\mathrm{thr(h)}]}$$

computed from 88 fish-only samples where thr(l) is the lower limit of the analysis (–60 dB in shallow layers and –62 dB in deep layers). It was chosen to be the highest threshold that still resulted in an asymptotic value of $s_a(f)$ based on the s_a versus threshold curve (Eckmann 1998). The highest and lowest possible thr(a) in the analysis were thr(h) – 2 dB and thr(l) + 2 dB, respectively.

Validation

The new method was validated with a secondary dataset: 48 samples throughout the open-water period having a negligible density of *Chaoborus* were analyzed using traditional analysis (threshold –60 or –62 dB) and with eq. 1. The accuracy of the method was tested by comparing the mean $s_a(f)$ value from eq. 1, $s_a(m)$, with the mean $s_a(f)$ value obtained through traditional analysis, $s_a(t)$, whereas the precision was evaluated based on the percent mean deviation (MD) of $s_a(m)$ from $s_a(t)$:

$$(3) \qquad \mathrm{MD} = \frac{\sum_{i=1}^{N}\left[100 \times \frac{|s_a(m) - s_a(t)|}{s_a(t)}\right]}{N}$$

where MD is the mean deviation for each thr(a) class, and N is the number of samples.

Comparisons were made for all thr(a) values applied in the present study to determine whether the practice of using transect-specific values, i.e., the lowest threshold eliminating *Chaoborus* in each transect, as thr(a) gave some gain in accuracy or precision. Otherwise a rational choice would be a constant threshold so high that reverberation is eliminated in all transects, because this would make the analysis easier.

Evaluation of the bias caused by *Chaoborus*

To evaluate possible overestimation of fish density if *Chaoborus* reverberation is not taken into account, we computed smelt densities for 3 and 16 August 1999 and 20 and 27 June 2000 using two approaches: (*a*) a traditional analysis in which thresholds were –60 or –62 dB, and (*b*) eq. 1. The surveys included in the analysis were selected to represent different phases in the seasonal development of the *Chaoborus* population. In June, the density of larvae is high in the water column, whereas in August, after their emergence, density is low (Liljendahl-Nurminen et al. 2002).

In these analyses, the $s_a(f)$ in each transect was converted to fish density by dividing it by the mean acoustic cross section (σ) of a single fish as determined by the trace-tracking option of EP 500 software (Lindem Data Acquisition). If the proportion of s_a from single fish echoes to total s_a was so low that σ could not be reliably computed, σ was computed based on length distributions in trawl catches and the length – target strength relationship determined for smelt in Lake Hiidenvesi (T. Malinen, unpublished data). Because of the negligible share of other species in the trawl catches (generally <1%), the density estimates were assumed to contain only smelt. The mean density estimates were computed as the weighted densities in transects, with transects' lengths as weights. The difference between density estimates was computed by subtracting the estimate given by method *b* from the estimate given by method *a*.

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Additionally, to estimate confidence limits for the difference between density estimates, we computed variance of mean difference, $\mathrm{Var}(\bar{d})$, considering the variation in length of transect (Shotton and Bazigos 1984):

$$\mathrm{Var}(\bar{d}) = \frac{\sum_{i=1}^{n}\left[(d_i - \bar{d})^2 l_i\right]}{\sum_{i=1}^{n} l_i (N-1)} \quad (4)$$

where l_i is the length of the ith transect, N is the number of transects, $\bar{d}$ is the mean difference, d_i is the difference between densities in transect i, such that $d_i = y_{a,i} - y_{b,i}$ (where $y_{a,i}$ is the density by method a in transect i and $y_{b,i}$ is the density by method b in transect i). Confidence limits were computed based on normal distribution.

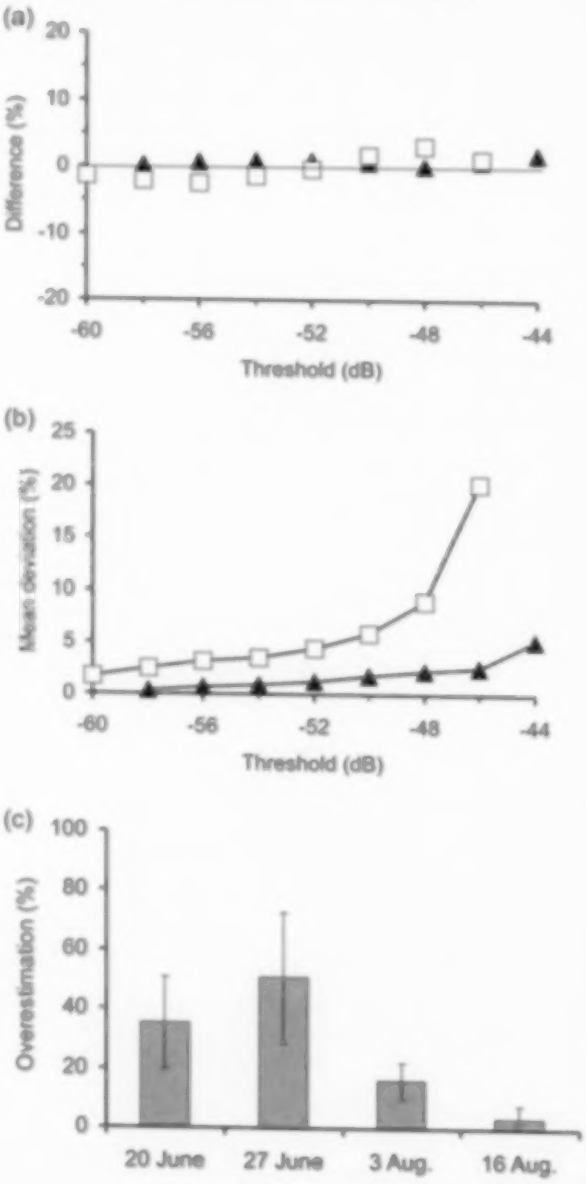
Results and discussion

Using the presented method, estimation of fish density was possible even when *Chaoborus* density was relatively high. According to net samples, *Chaoborus* densities in the water column varied between 1000 and 6000 individuals·m^{-2} (Liljendahl-Nurminen et al. 2002). Considering the depth distribution of larvae, the highest *Chaoborus* densities successfully eliminated were between 200 and 300 individuals·m^{-3}. Only a few metalimnetic aggregations of *Chaoborus* in July were so dense that elimination failed, because *Chaoborus* backscattering extended beyond the highest threshold included in the analysis.

Given the traditional analysis as an unbiased method in the validating samples, eq. 1 gave accurate estimates of $s_a(f)$. On average, it gave only 0.8% higher and 0.2% lower $s_a(f)$ values than the traditional analysis in shallow and deep layers, respectively. The difference between means with given thr(a) varied between –2.5% and +3.5%, and no noticeable trend existed (Fig. 2). The mean deviation increased with thr(a) in both depth layers (Fig. 2). In the shallow layer, MD was less than 3% with thr(a) up to –46 dB and increased only slightly. In the deep layer, however, MD increased rapidly after –52 dB, up to 20% with the highest thr(a). This suggests that the applied practice of choosing the lowest threshold eliminating reverberation yielded a remarkable gain in precision of the estimate of $s_a(f)$.

Because the developed method gave practically unbiased results in the validating samples, it could be used as a reference value for traditional analysis when evaluating the bias caused by *Chaoborus*. The analyses revealed that the bias can be high if the threshold is chosen to contain all $s_a(f)$ while *Chaoborus* reverberation is not taken into account. The degree of overestimation of fish density varied widely between seasons: in June, it was as high as 35–55%, whereas in August, it was only 3–16% (Fig. 2). The high overestimation in June was most likely caused by the high *Chaoborus* density in the water column and overlapping distributions of *Chaoborus* and fish. Lower bias in August was understandable, because *Chaoborus* density in the water column collapsed because of emergence. In addition, larvae were smaller (mean length 6–7 mm) in August than in June (9–10 mm; Liljendahl-Nurminen et al. 2002).

Fig. 2. (*a*) The percent difference between actual s_a (traditionally computed) and s_a computed with eq. 1 as a function of thr(a) in fish-only validating samples in shallow (▲) and deep (□) layers. A negative value indicates that actual s_a is greater than computed, and a positive value indicates that it is lower than computed. (*b*) Mean percent deviation between actual s_a with traditional method and s_a by eq. 1 as a function of thr(a) in shallow (–▲–) and deep layers (–□–) in validating samples. (*c*) The overestimation of smelt density estimate (older than young-of-the-year) by traditional analysis resulting from reverberation by *Chaoborus* in June 2000 and August 1999. The mean smelt density (older than young-of-the-year) was 8000 individuals·ha^{-1} in June and 12 000 – 14 000 individuals·ha^{-1} in August.



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In theory, fish density might be estimated with the presented method even in very high *Chaoborus* densities by increasing thr(h) and thr(a) in the analysis. However, in Lake Hiidenvesi, this was not possible because a higher thr(h) produced unacceptably large variation in p values because of patchy distribution of smelt schools. If schools were hit in a sampling unit, very high p values were observed, whereas scattered smelts produced only low p values. Modeling this mechanism is complicated, because the variation in size and density of smelt schools must be wider than that between individual fishes. However, the use of very high thresholds may be unnecessary for practical purposes, because the ability of *Chaoborus* to escape fish (McQueen et al. 1999) should ensure that high densities of larvae and fish do not occur in the same layer. This was also observed in this study: the trawl catch of smelt from the layer having densest swarms of *Chaoborus* was only 2% of that in the epilimnion.

The method is applicable to other lakes, though this is not an effortless transition, because thr(a), thr(h), and p values should be determined for each specific case. In addition, care should be taken when applying the method elsewhere: in Lake Hiidenvesi, the fish assemblage is dominated by one species showing only a small variation in size (total length 4.5–8.5 cm). In the length and target strength (TS) distributions, typically only two modes exist (in August, for instance, young-of-the-year at 5 cm and –52 dB, older at 8 cm and –47.5 dB) and TS distributions are highly overlapping. More variation in p values might occur if several species or size groups were present. Additionally, if the growth rate of fish is higher, p values should be determined separately for various seasons. Moreover, p values and appropriate thr(h) values are probably functions of the schooling behavior of the species. In the present study, the value of thr(h) was chosen to be so low that the effect of fish schools was eliminated. In multispecies assemblages, however, this may be more difficult. Because the p values may also be instrument-dependent, influenced by, for example, frequency and beam pattern of the transducer, the universality of the s_a versus threshold relationship might be a valuable area for further research. The presented method might also be able to eliminate reverberation resulting from other unwanted targets, because it is based on the acoustic properties of fish rather than those of unwanted targets.

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Spatial dynamics of female snow crab (*Chionoecetes opilio*) in the eastern Bering Sea

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Abstract: Snow crab (*Chionoecetes opilio*) migrations in the eastern Bering Sea have long been ignored. Based on preliminary information, we hypothesized that females undergo an extensive ontogenetic migration, tracking down environmental gradients. We analyzed a 25-year time series of survey data and defined ontogenetic stages in terms of a "shell condition index" calibrated with radiochemical methods. "Pseudo-cohorts" of mature females (groups of females that undergo puberty molt in a given year) "recruit" to the mature female pool in the Middle Domain (50–100 m) of the intermediate shelf. Females undergo puberty molt and primiparous mating in winter. Over the next year, they migrate an average net distance of 73.5 nautical miles towards the shelf edge following a predominantly northeast–southwest direction. Maximum post-terminal molt life span is 6–7 years. Results support the hypothesis that the variable tracked is near-bottom temperature. Although near-bottom temperature fields vary from year to year, the corresponding vector field is a conservative template, which explains the consistency observed in the pattern of migration. Elucidation of the life history schedule of mature female snow crabs in the eastern Bering Sea revealed that it is very similar to that reported for eastern Canada, although patterns of migration may differ substantially between the two systems.

Résumé : L'étude des migrations du crabe des neiges (*Chionoecetes opilio*) dans l'est de la mer de Béring a longtemps été négligée. Sur la base de données préliminaires, nous posons l'hypothèse selon laquelle les femelles complètent une importante migration ontogénique, en suivant des gradients environnementaux. Nous avons analysé une série temporelle de 25 années de données d'inventaire et nous avons défini les stades ontogéniques d'après un « indice de condition de la carapace » calibré au moyen de méthodes radiochimiques. Des « pseudo-cohortes » de femelles matures (des groupes de femelles qui subissent la mue de puberté dans une même année) s'incorporent comme « recrues » dans le pool de femelles adultes dans le domaine central (50–100 m) de la plate-forme intermédiaire. Les femelles complètent leur mue de puberté et leur accouplement de primipare au cours de l'hiver. Dans l'année qui suit, elles migrent sur une distance moyenne nette de 73,5 milles marins vers la marge de la plate-forme, en s'orientant dans une direction surtout nord-est et sud-est. La longévité maximale après la mue terminale est de 6–7 ans. Nos résultats appuient l'hypothèse qui veut que la variable qui est suivie soit la température près du fond (NBT). Bien que les champs de NBT varient d'une année à l'autre, le champ vectoriel qui en résulte a une structure relativement stable, ce qui explique la constance qui existe dans les patrons de migration. L'élucidation du calendrier du cycle biologique des crabes des neiges femelles matures dans l'est de la mer de Béring montre qu'il est très semblable à celui qui a été signalé dans l'est du Canada, bien que les patrons de migration puissent différer considérablement entre les deux systèmes.

[Traduit par la Rédaction]

Introduction

Over recent years, there has been considerable interest in exploring the spatial dimensions of population dynamics and of the fishing process (Kruse et al. 2001), which are of particular significance in the case of benthic organisms (Orensanz and Jamieson 1998). The implications of ignoring the spatial structure of benthic stocks, a usual simplification made in model applications under the unit stock paradigm and the dynamic pool assumption (Caddy 1975), need to be better understood. The snow crab (*Chionoecetes opilio*) fishery of the eastern Bering Sea (EBS) is an interesting case in point.

Snow crabs are distributed over the extensive shelf of the EBS (Fig. 1), where it has supported one of the largest crab fisheries in the world (Otto 1989; Stevens et al. 2002). Annual harvest quotas are calculated on the basis of aggregated estimates of abundance using data from systematic summer trawl surveys that have been conducted by the US National Marine Fisheries Service (NMFS) since 1975 (Stevens et al. 2002). The snow crab population of the EBS is assessed and managed as a unit stock (Zheng et al. 2002), although spatial

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Fig. 1. Presence of snow crabs (*Chionoecetes opilio*) in the eastern Bering Sea based on the 1975–2001 NMFS trawl surveys. Dots represent stations where snow crabs were caught at least once. The thin lines correspond to the 50-, 100-, and 200-m isobaths.

effort allocation in the male-only fishery (nowadays restricted to the winter months) is constrained by ice cover. The less mobile the individuals and (or) the more spatially structured the population, the less appropriate this assessment/management framework would be. It has been traditionally held that snow crab migrations in the EBS are negligible, if present at all. Somerton (1981*a*, p. 170) concluded that "short-term changes due to crab migration appear not to be important", and Incze et al. (1987, p. 1143) pointed out that here is no indication that either [snow or Tanner crab] undertake large seasonal migrations in this region. Yet, paradoxically, differential patterns of distribution of snow crab life history stages in the EBS have long been recognized. Data from the trawl surveys show a striking pattern, early noticed by Somerton (1981*b*): some areas contain many immatures but few adults and vice versa. The distribution of different types of females shows an offshore shift in location along a progression of life history stages, suggesting a large-scale ontogenetic female migration with a northeast–southwest orientation (Orensanz and Armstrong 1997; Zheng et al. 2001, their fig. 1). The same may apply to males, but this is less clear because of the fact that most of the data do not discriminate between morphologically immature and mature males. The determinants of the hypothetical migration have not been investigated.

Understanding the spatial dynamics of female snow crabs has significant implications for conceptual and formal models of stock dynamics. Reproductive success of the mature female population is not simply related to the size of the spawning stock but also to (*i*) the relative spatial distribution of mature males and females and (*ii*) females hatching their eggs at locations that, given the pattern of water circulation, are appropriate as potential "sources".

Most studies dealing with the distribution of benthic organisms in relation to environmental variables focus on the absolute magnitude of those factors. The geographic distribution of the variables is usually represented in the form of isolines or contour plots (e.g., Somerton 1981*a*), and the relationship between environmental factors and organisms has been investigated by means of correlation analysis, multivariate methods, generalized additive models (Swartzman et al. 1992), cumulative distributions along environmental axes (Perry and Smith 1994), etc. Spatial gradients have received little attention, although vector fields are the "roadmaps" that individual crab "see" while trekking over the seabed. An individual that settles away from locations best suited to its environmental preferenda may never reach those locations if near-bottom gradients do not lead it in the right direction. The geographic distribution of environmental variables and their aggregate association with abundance are insufficient to understand the patterns of spatial distribution of slow-moving benthic marine organisms.

This study deals with the life history of females after their terminal molt, once they have "recruited" to the mature pop-

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ulation and stop growing. Crabs cannot be aged, and the females molting into maturity in any given year are likely to belong to more than one year class. Thus, correspondence between year classes and cohorts of females entering the mature pool cannot be established with certainty. To avoid confusion, we refer to all female crabs entering the mature pool during a given year as a "pseudo-cohort". Here, we present strong evidence indicating that female pseudo-cohorts undergo an ontogenetic migration in the EBS, hypothesize that this movement is accomplished by following conservative patterns of near-bottom environmental gradients, and explore ways to depict the corresponding gradient fields.

Study system

EBS shelf

The extensive shelf of the EBS, more than 500 km wide, ends seawards in a shelf break (generally coincidental with the 200-m isobath) that spreads along the northwest–southeast direction; the two main geographical features are the St. Matthew and Pribilof islands (Fig. 1). The shelf is conveniently partitioned into three sectors (Schumacher and Stabeno 1998, their fig. 27.1): northern, central, and southeastern. The latter two, ranging (combined) from the Alaska Peninsula in the southeast to north of St. Matthew Island, constitute the geographic setting of our study. Three zones or domains are usually recognized (Schumacher and Stabeno 1998): Inner or Coastal (shoreward from the 50-m isobath), Middle (between the 50- and 100-m isobaths), and Outer (between the 100-m isobath and the shelf break). The Outer Domain is cut into two naturally discrete segments (southeast and northwest) by the Pribilof Canyon. The Pribilof and St. Matthew islands are located within the Middle Domain; their surrounding shallows are considered here as part of the Middle Domain even though depth is less than 50 m. Up to 75% of the EBS shelf water is covered by ice in late fall – early spring. The advance and retreat of ice average about 1700 km north–south and is the most extensive such process in Arctic regions (Niebauer 1999).

Circulation in the EBS is dominated by the Alaskan Coastal Current, which enters from the Gulf of Alaska through Unimak Pass and other passes along the Aleutian Island chain (Reed and Stabeno 1999) and moves east onto the EBS shelf as a cyclonic gyre. Much of the Alaskan Coastal Current that enters through Unimak Pass flows to the northwest (Bering Slope Current); a second branch slowly flows counterclockwise within the Coastal Domain, first along the north side of the Alaskan Peninsula, then across the mouth of Bristol Bay, and finally to the north. Long-term average speeds are relatively high within the Bering Slope Current (5–20 $cm \cdot s^{-1}$), slower nearshore along the Peninsula (1–5 $cm \cdot s^{-1}$), and very weak within the Middle Domain (Kinder and Schumacher 1981), where there is virtually no net directional flow. Oceanographic and satellite data have revealed an eastward flow (2–3 $cm \cdot s^{-1}$) across the shelf north of the Pribilof Islands (Schumacher and Stabeno 1998). Flow over the Outer Domain is moderate (4–8 $cm \cdot s^{-1}$), following the 100-m isobath towards the northwest.

The southeastern sector of the shelf is divided by a series of three fronts into two interfrontal zones that correspond to the Middle and Outer domains (Coachman 1986). Many biological phenomena can be conveniently conceptualized in this framework (Iverson et al. 1979), although the location and dynamics of the fronts are not clearly established for the Central Shelf (Schumacher and Stabeno 1998). As ice melts in spring, the less saline water "floats" over higher salinity marine water, and this upper layer is heated, which results in stratification of the water column. Crab larvae develop in the upper layer where higher temperature and greater food production benefit growth rate (Incze et al. 1987). The stratified water column "traps" an extensive "cold pool" layer (water <2 °C) down to the seafloor over a large area of the EBS. In summer, this cold pool averages 200 km north–south and 500 km east–west (Wyllie-Echeverria and Wooster 1998). Following a warm winter, there may be virtually no cold pool over the EBS – Bristol Bay region (e.g., 1979), or the cold pool may cover most of this region following a very cold winter (e.g., 1995) (B. Ernst, J.M. Orensanz, and D.A. Armstrong, unpublished data). The cold pool may persist over larger or smaller areas of the EBS for several consecutive years (Loher 2001), and the degree of spatial coverage influences the abundance and distribution of fishes and invertebrates.

Ontogeny and reproductive ecology of female snow crab in the EBS

Following Alunno-Bruscia and Sainte-Marie (1998), we use the terms "immature" for sexually immature females, "prepubescent" for females in the instar previous to puberty (terminal) molt, and "mature" for postpuberty females. Females stop growing after the puberty molt, when they reach adulthood; somatic growth is primarily an aspect of the immature life history period. At the puberty molt, there is a dramatic, readily recognizable change in the relative width of the abdominal flap or pleon. Because the molt to maturity is terminal, the best depiction of size at maturity is the size–frequency distribution of mature females (Somerton 1981*a*). Grand mean carapace width (CW) of mature female snow crab was estimated by Otto (1998) at 56 mm for the EBS (data from 1989–1994 NMFS surveys); most mature females are in the range 35–80 mm (exceptionally down to 26 mm or up to 89 mm). Mean size of mature females varies geographically in the EBS. Somerton (1981*b*), based on data from the 1979 NMFS survey, explored geographical gradients, finding that mean size decreases northward from approximately 70 mm at 55°N to only 40 mm at 63°N. Otto (1998), based on 1989–1994 NMFS survey data, and Zheng et al. (2001), based on 1978–1999 NMFS survey data, showed the same basic pattern. Instar and age at maturity have not been identified in the EBS. In eastern Canada, females reach maturity at instars ranging from IX to XI, rarely VIII (Alunno-Bruscia and Sainte-Marie 1998; Comeau et al. 1998); maturity is reached at 4.5–6.5 years postsettlement age (Alunno-Bruscia and Sainte-Marie 1998) in the Gulf of St. Lawrence and at 7–8 years in Bonne Bay, Newfoundland (Comeau et al. 1998). The two size modes illustrated by Somerton (1981*b*) may correspond to instars IX and X or X and XI, implying that in the EBS, most females grow through seven to nine immature instars and the prepubescent instar before maturity. Mating behavior and ecology of snow crabs are very complex and have received considerable attention

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(Elner and Beninger 1995). Experimental work conducted in eastern Canada has demonstrated that females can mate after the puberty molt while still in a soft-shell condition or (in subsequent years) while in a hard-shell condition; presumably, this is the case in the EBS as well. The first are termed "primiparous" and the second "multiparous". The calendar of reproductive events is not well established for the EBS. Hatching/oviposition (and by implication multiparous mating) starts in March and is completed by July (Somerton 1981*b*, p. 32).

Materials and methods

Survey data

Time series of data were collected during trawl surveys conducted by the NMFS between 1975 and 2001 (Otto 1998; Stevens et al. 2002). Surveys follow a systematic sampling design, where stations are regularly spaced over a 20 nautical mile (nmi) × 20 nmi grid and sampled every year. The 1975–1977 surveys were not included in the analyses because they covered only the southern end of the geographic range of interest. The systematic sampling surveys had a consistent spatial coverage after 1978 (Fig. 1). The core temporal window of the surveys is June–July, but in some years, work started in May (1975–1981 and 1999–2000) or ended in August (1975–1976, 1978–1979, 1982–1986, and 1988–1992). Near-bottom temperature (NBT) has been regularly recorded since the initiation of the survey program. Several observations were made on each individual caught in a haul or from a subsample when the catch was too large ("Manual of crab sampling methods for the trawl survey in the eastern Bering Sea"; available from NMFS, Alaska Fisheries Science Center, P.O. Box 1638, Kodiak, AK 99615-1638, USA). In the case of females, these included CW (millimetres), shell condition (see next section), sexual maturity (immature/mature), and various related reproductive variables.

Shell condition index (SCI)

After terminal molt, condition of the shell changes as time passes owing to wear and colonization by epibionts. Subjective SCIs have been devised as a rough correlate of shell age. We used the SCI categories routinely utilized by NMFS as follows. (0) Ready to molt or molting; (1) "soft shell": carapace soft and pliable, duration very short (weeks); (2) "new hard shell": carapace firm to hard, clean, brick red to yellow brown on the topside; (3) "old shell": carapace hard, topside usually yellowish brown, thoracic sternum and underside of legs yellow with numerous scratches, pterygostomial and branchial spines worn and polished, spines on the meri and metabranchial region rounded, epifauna (barnacles and leech cases) usually (but not always) present; (4) carapace hard, topside yellowish brown to dark brown, thoracic sternum and undersides of legs yellow with many scratches and dark stains, pterygostomial and branchial spines rounded with the tips sometimes worn off, dactyli very worn, sometimes flattened at the tip, spines on meri and metabranchial region worn smooth, sometimes completely gone, epifauna most always present (large barnacles and bryozoans); (5) conditions observed in 4 much advanced, large epifauna almost completely covers the crab, carapace is worn through in metabranchial regions, along pterygostomial branchial spines, or on the meri, dactyli flattened, sometimes worn through, mouth parts and eyes sometimes immobilized by barnacles. Categories 4 and 5 are collectively labeled as "very old shell" and sometimes subsumed under the old shell category for reporting. We refer to this pool as "4*". Females in SCI 0 and SCI 1 (ready to molt or molted just before capture) are very uncommon in the surveys (on average 0.26% of all mature females) and were not included in our analyses.

For the radiochemical calibration of SCI as an index of time past terminal molt (see section below), we introduced two subcategories: SCI 0* for crabs with extremely soft bodies and SCI 2* for crabs classified as SCI 2 that were closest to conditions defining SCI 3. SCI 0*, the softest crabs caught (intermediate between categories 0 and 1), are valuable because they allow validation of assumptions on initial conditions made in radiometric calculations. While it is reasonable to assume that SCI 2 crabs molted within recent months, SCI 2* crabs are expected to help put an upper bound on the duration of that period.

Radiochemical calibration of SCIs

The results presented here (Table 1) correspond to an extension of a previous study by Nevissi et al. (1996) to which we refer for information about field collection and laboratory methods. The estimation is based on the determination of naturally occurring $^{228}Th/^{228}Ra$. The basic assumptions for radiometric age determination are that (*i*) during molting, virtually all of the calcium and associated nuclides are lost by the animal and that (*ii*) the new carapace is calcified rapidly enough after molting so that (*iii*) addition or removal of radionuclides during the intermolt period is negligible. When the new carapace is formed, radium is incorporated with calcium into the exoskeleton and with time declines to thorium:

$$(1)\quad {}^{228}\mathrm{Ra} \xrightarrow[5.76\ \text{years}]{\beta} {}^{228}\mathrm{Ac} \xrightarrow[0.15\ \text{h}]{\beta} {}^{228}\mathrm{Th} \xrightarrow[1.91\ \text{years}]{\alpha} {}^{228}\mathrm{Ra}$$

^{228}Th accumulates with time and the activity of ^{228}Th to ^{228}Ra will lead to an age estimate according to

$$(2)\quad \frac{A_{^{228}\mathrm{Th}}}{A_{^{228}\mathrm{Ra}}} = 1.4925(1 - e^{-0.2444t})$$

where t is time expressed in years.

All of the data ($n = 21$) correspond to male individuals, including 17 specimens of snow crab and four specimens of the closely related Tanner crab (*Chionoecetes bairdi*). Assignment of specimens to SCI categories followed consultation with the NMFS expert onboard during the 1992 survey (Dr. Brad Stevens, US National Marine Fisheries Service, P.O. Box 1638, Kodiak, AK 99615-1638, USA). All of the specimens analyzed were males, and the data are utilized under the presumably mild assumption that shells of males and females deteriorate at a similar rate over time. One of the reasons to do measurements on males is that females in SCI 1 are almost never caught in the trawl surveys, but that stage is very important in the calibration of SCIs.

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Table 1. Radiometric estimates of shell age in male snow crabs (*Chionoecetes opilio*) and Tanner crabs (*Chionoecetes bairdi*) ($n = 21$) collected in the eastern Bering Sea during the NMFS survey of 1992.

Shell condition	CW (mm)	Age (years)	Error (years)	Coordinates	Depth (m)	Species
0*	121	0.05	0.26	59°20′N, 171°49′W	43	*C. opilio*
0*	110	0.11	0.27	59°20′N, 171°49′W	43	*C. opilio*
0*	132	0.11	0.19	59°20′N, 171°49′W	43	*C. opilio*
1	118	0.15	0.26	59°20′N, 171°49′W	43	*C. opilio*
1	130	0.23	0.27	59°20′N, 171°49′W	43	*C. opilio*
1	116	0.25	0.24	59°20′N, 171°49′W	43	*C. opilio*
2*	93	0.33	0.28	57°00′N, 167°43′W	42	*C. bairdi*
2*	122	0.42	0.26	57°00′N, 167°43′W	42	*C. bairdi*
2*	97	0.66	0.30	59°00′N, 171°47′W	46	*C. opilio*
2*	123	0.78	0.32	59°00′N, 171°47′W	46	*C. opilio*
2*	121	0.85	0.27	57°00′N, 167°43′W	42	*C. opilio*
2*	66	1.07	0.29	59°00′N, 171°47′W	46	*C. opilio*
3	117	0.92	0.34	59°00′N, 171°47′W	46	*C. opilio*
3	69	1.04	0.28	59°00′N, 171°47′W	46	*C. opilio*
3	78	1.10	0.30	59°00′N, 171°47′W	46	*C. opilio*
4	100	4.43	0.33	57°21′N, 167°45′W	39	*C. opilio*
4	93	4.89	0.37	58°20′N, 171°38′W	52	*C. bairdi*
4	100	6.60	0.33	57°00′N, 167°43′W	42	*C. opilio*
5	111	2.70	0.44	58°60′N, 169°12′W	28	*C. opilio*
5	100	4.21	0.34	59°00′N, 171°47′W	46	*C. bairdi*
5	110	6.85	0.58	58°60′N, 169°12′W	28	*C. opilio*

Analysis of NMFS survey data

Index of abundance

Catch per haul was used to estimate density using swept-area methods (Sparre and Venema 1998). Average density for each station was estimated as

$$(3) \qquad D_t^S = \frac{1}{n_t^S} \sum_{i=1}^{n_t^S} \frac{d_{i,t}^S}{A_{i,t}^S}$$

where D_t^S is density at station S in year t, n_t^S is the total number of hauls for station S in year t, $A_{i,t}^S$ is the area swept by haul i in station S and year t, and $d_{i,t}^S$ is the number of crabs caught by haul i in station S and year t. We refer to this as "relative abundance" because no gear efficiency correction was used in the analysis. The original database contains approximately 200 000 mature female records.

Parsing and graphics

The data were parsed and analyzed using scripts in AWK stream editor (Robbins 2001). Graphical analysis was conducted using Generic Mapping Tools graphical software (Wessel and Smith 1998). Some graphs required surface plots, which implied the additional calculation of regular station grids over the entire distributional area. This was accomplished by using a near-neighbor algorithm.

Calculation of centroids

Centroids of density for each ontogenetic group in a particular year were computed as weighted averages of longitudinal and latitudinal components:

$$(4) \qquad C_t^\Phi = \frac{\sum_{S}^{n_t^T} \Phi_t^S D_t^S}{\sum_{S}^{n_t^T} D_t^S}$$

and

$$(5) \qquad C_t^\Theta = \frac{\sum_{S}^{n_t^T} \Theta_t^S D_t^S}{\sum_{S}^{n_t^T} D_t^S}$$

where C_t^Φ is the latitudinal component of the density centroid in time t, C_t^Θ is the longitudinal component, n_t^T is the total number of stations for year t, Φ_t^S is the average longitude of station S (across hauls) in time t, and Θ_t^S is the average latitude of station S (across hauls) in time t.

Angle and distance of migration

The geographical location of pairs of centroids of consecutive life history stages allowed us to estimate time series of mean migration distances and angles of migration relative to the north for specified pairs of stages (e.g., mature females in SCI 2 in year i and SCI 3 in year $i + 1$; see the Results section for rationale):

$$(6) \quad \tau_t = [({}^{\mathrm{SCI}\,2}C_t^\Phi - {}^{\mathrm{SCI}\,3}C_{t+1}^\Phi)^2 + [\cos(0.5\,\mathrm{ABS}({}^{\mathrm{SCI}\,2}C_t^\Theta - {}^{\mathrm{SCI}\,3}C_{t+1}^\Theta))({}^{\mathrm{SCI}\,2}C_t^\Phi - {}^{\mathrm{SCI}\,3}C_{t+1}^\Phi)]^2]^{1/2}$$

for southeast migration

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$$(7)\quad \Gamma_t = 180° - \text{arctg}[\{\cos(0.5\,\text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1}))\times \text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1})\}/\text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1})]$$

for southwest migration

$$(8)\quad \Gamma_t = 180° + \text{arctg}[\{\cos(0.5\,\text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1}))\times \text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1})\}/\text{ABS}({}^{\text{SCI 2}}C^{\Phi}_{t} - {}^{\text{SCI 3}}C^{\Phi}_{t+1})]$$

where τ_t is the average migration distance from time t to time $t + 1$ and Γ_t the average angle of migration with respect to true north from year t to year $t + 1$.

Mean size at maturity

Mean values were computed as a weighted average by sampling station (weights proportional to density of each size class in each sample). Equal weight was given to the average size at maturity per station when integrating over time. Size selectivity of the survey gear has not been assessed; immature females smaller than 35 mm CW are poorly represented in the samples. It is assumed that vulnerability is the same for all mature females.

Mapping of environmental gradients

Temperature and depth information was also retrieved from the NMFS trawl survey data set. Given our interest in gradient information, we transformed the original data (temperature and depth) into gradient (vector) fields. To that end, we followed three steps. First, data points for gradient calculation were selected based on the full complement of eight associated observations. Second, overall directional latitudinal and longitudinal components were estimated at each point with respect to the eight immediate neighbor stations in the grid. Third, angle and magnitude of the gradients were computed from vectorial north–south and east–west components.

Results

Aggregated pattern of geographical distribution

Records of snow crab in the NMFS survey data cover virtually the entire grid surveyed between 1978 and 2001 in the EBS shelf, ranging from Bristol Bay and off the Alaska Peninsula to the vicinity of St. Lawrence Island (Fig. 1). The aggregated spatial distribution of females (1978–2001 surveys, data pooled) indicates, however, that significant abundance of mature females has been historically concentrated in the Middle and Outer domains of the Central Shelf (Fig. 2). The boundaries of the aggregated distribution are well captured to the east, west, and south, but the female stock extends farther to the north and northwest over the North Shelf (Paul et al. 1997). The latter has not been regularly surveyed.

The aggregated pattern (1978–2001, data pooled) varies for females in different life history stages. Immature females are remarkably concentrated in the Middle Domain of the Central Shelf and adjacent fringe sectors of the Coastal Domain with hotspots around St. Matthew Island, while mature females in SCI 2 (Fig. 2*b*) (largely primiparous) are spread over the same region plus the shallower fringes of the Outer Domain. Females in SCI 3 and SCI 4+ (Figs. 2*c* and 2*d*) (mostly multiparous) are progressively displaced towards the Outer Domain.

The aggregated distribution of mature females (Figs. 2*b*–2*d*) shows two broad clusters, roughly separated by the 59°N parallel, each containing a variable number of local maxima. The latter reflect localized pulses of abundance (recruitment to the mature population in the case of SCI 2 females) in particular years. The southern cluster is distributed to the east, north, and northwest of the Pribilof Islands, the southern half of the Central Shelf. The northern cluster is distributed in the northern part of the Central Shelf around St. Matthew Island, where the Outer Domain expands to the west. In some cases, we analyze the two clusters separately using the 59° N parallel as a convenient and reasonable (albeit somewhat arbitrary) line for the partition.

Trends in recruitment to the mature population

By the time of the year when the surveys were conducted, all pubescent females had undergone their terminal molt to primiparous status, and almost all multiparous females were carrying a new clutch of eggs. This conclusion is based on two facts. First, out of a total of 195 366 mature females sampled and checked between 1975 and 2001, 98.5% had yellow/orange eggs. Virtually all SCI 2 egg-carrying females had yellow/orange eggs (0.24% had brown eggs and 0.31% were not carrying eggs). Second, females in SCI 1 were reported in very low numbers during the surveys. Given that the NMFS surveys are conducted once the puberty molt season is over, the abundance of females in SCI 2 reflects the annual recruitment to the mature female population. These females constitute a cohort, but we prefer to use here the term "pseudo-cohort" because the term "cohort" is often equated with "year class". We did not attempt to trace pseudo-cohorts back into the immature female pool because the latter is possibly a mixture of individuals in different instars that may recruit into the mature population over more than one year.

The trend in pseudo-cohort strength shows well-defined pulses in the two clusters (north and south sectors of the Central Shelf) during the period 1975–2001 (Fig. 3). Recruitment rose to a maximum in 1980 and then dropped to a minimum in 1984 in both clusters. Recruitment was stronger in the south cluster before 1983, but the relationship reversed afterwards, remaining generally stronger in the north cluster during the 1983–2001 period. During this period, although there is some coherence in fluctuations between the two clusters, there are also noticeable differences. During the late 1990s, recruitment dropped sharply in both regions but recovered in 2000–2001 in the north while remaining close to zero in the south during the 5-year period 1997–2001. While peaks in recruitment generally coincide between regions after the 1985 minimum, peaks in the south are dominated by a single pseudo-cohort (1988, 1992, and 1994), while in the north, they are spread over 2 or 3 years (1987–1988 and 1993–1995, respectively). This could reflect differences in year-class strength and (or) differences in age at maturity between the two regions. Although deserving further research, this subject is beyond the scope of our study.

Calibration of shell condition

Females in SCI 2 (virtually all carrying yellow/orange eggs) are presumed to be primarily primiparous, having molted a few months before the survey. Past SCI 2, the meaning of

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Fig. 2. Three-dimensional illuminated surface plots of female snow crab (*Chionoecetes opilio*) relative abundance by life history stage: (*a*) immature, (*b*) SCI 2, (*c*) SCI 3, and (*d*) SCI 4+, all integrated over time from 1978 to 2001. In the two-dimensional panels at the bottom, shading is proportional to density. Isobath lines are 50, 100, and 200 m.

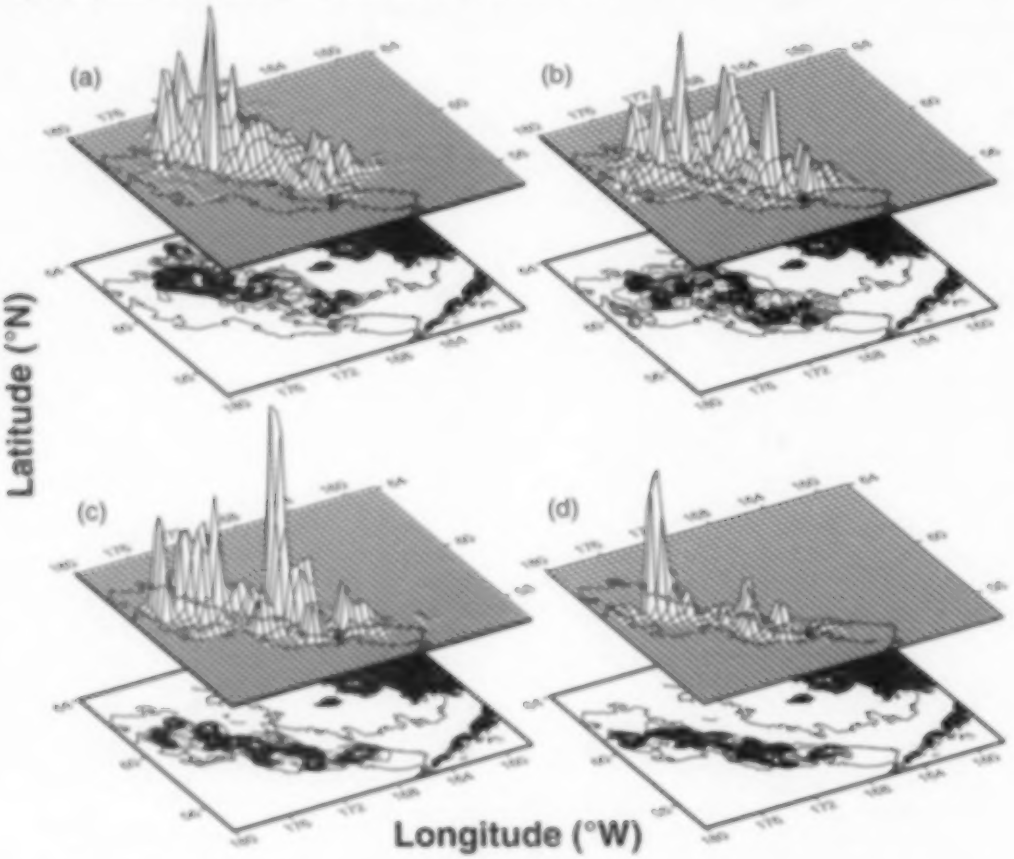
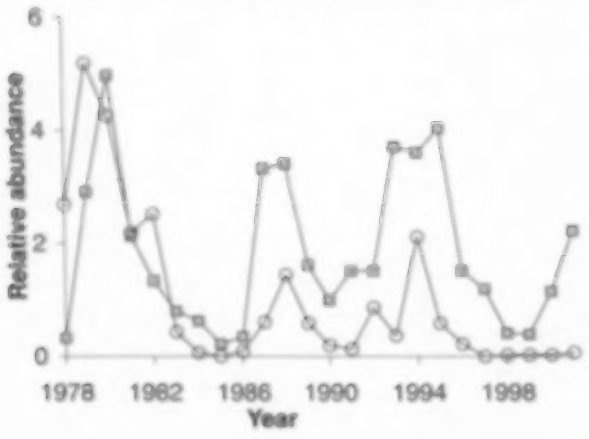


Fig. 3. Recruitment to the mature female snow crab (*Chionoecetes opilio*) population north (shaded squares) and south (open circles) of 59°N in the eastern Bering Sea expressed as an index of abundance of females in SCI 2 during the summer NMFS surveys.



the SCI is unclear. We calibrated it as an index of time past puberty molt in two different ways.

Radiochemical methods

The radiometric technique indicated a postmolt life of 1 month on average (n = 3) for male crabs in SCI 0+. Considering the measurement error surrounding the estimates (Table 1), the conclusion (consistent with our expectation and assumptions) is that crabs collected in SCI 0+ had molted very recently (down to the day when the sample was taken). Estimated postmolt life of SCI 1 males was 1.8–3 months (2.5 months on average, n = 3) (Table 1). Since the males sampled were morphologically mature, this implies that the male terminal molt extends approximately from March through May. Our results support assumptions about the initial condition made in radiometric calculations. Radiometric ages calculated for the oldest looking crab that would be classified as SCI 2 by an expert ranged between 4 months and 1 year, with a mean radiometric age of 8.2 months (Table 1). Considering that (*i*) during the period of the surveys (May–August), females were very rarely found in SCI 1, (*ii*) the condition SCI 2+ was observed in males but not in females (SCI 2 females were mostly on the "new-looking"

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end of the SCI 2 range), and (*iii*) the minimum radiometric age of SCI 2* was 4 months, we conclude that the SCI 2 category corresponds to primiparous females that molted (on average) 3–4 months before the survey. This indicates that female terminal molt and primiparous mating in the EBS occur during the winter (February–March).

The radiometric age of three SCI 3 males collected in June was, on average, 1 year (11–13 months) (Table 1; Fig. 4*a*), which puts their last molting event slightly after the inferred peak of the male terminal molt season (March–May). Shells of females in SCI 3 should be slightly older, approximately 1.5 years on average, which implies that they correspond to the first-time multiparous group assuming an annual reproductive cycle. The radiometric age of shells in SCI 4 and SCI 5 ranges from 2.7 to 6.8 years. Our results suggest that SCI 4 and higher are poorly correlated (if at all) with shell age (Fig. 4*a*). Since crabs in SCI 5 included some of the ones in the poorest condition collected during the 1992 survey, it is reasonable to conclude that maximum life span after terminal molt is on the order of 6–7 years.

Tracking an isolated pseudo-cohort

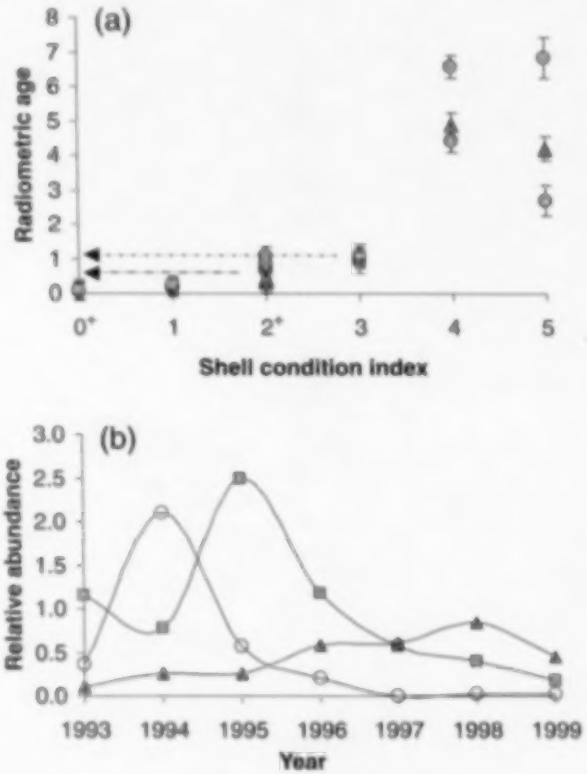
One possible way of calibrating the SCI is to track the abundance of females in each SCI over a few years after the recruitment of a strong pseudo-cohort. Ideally, this should not be immediately preceded or followed by other strong pseudo-cohorts so that transitions can be clearly established. The 1994 pseudo-cohort in the southern cluster best satisfies these criteria: it was preceded by a much weaker pseudo-cohort and, more importantly, was followed by 7 years of insignificant recruitment (Fig. 4*b*). A clear peak in the abundance of females in SCI 2 in 1994 was followed 1 year later (1995) by a peak of females in SCI 3. The abundance of SCI 4 females gradually increased between 1996 and 1998, declining afterwards. Abundance of females in SCI 5 was negligible throughout the period. This pattern is consistent with the hypothesis that the transition between SCI 2 and SCI 3 takes 1 year. Females in SCI 4 appear to comprise a pool of individuals that were mostly 2 or more years past their puberty molt.

Tracking of an individual pseudo-cohort and radiometric data both suggest that females in SCI 2 and SCI 3 are definable in terms of time past the puberty molt but that females in SCI 4 and SCI 5 correspond to a pool of females that molted 2–6 years before. Thus, in subsequent analyses, we consider only three groups: SCI 2, SCI 3, and SCI 4*. These are assumed to have had their terminal molt, respectively, shortly before the time of the survey, 1 year earlier, or 2 or more years before. Given the very low relative abundance of mature females in SCI 4*, natural mortality after the second year past terminal molt must be very high; most SCI 4* females are likely to be only 2 years past terminal molt.

Shifts in the geographic distribution of pseudo-cohorts

In a preceding section, we illustrated geographical shifts in the distribution of pooled mature females along the progression of SCI categories (Fig. 2). These shifts are better appreciated when individual pseudo-cohorts are tracked over time, as illustrated with the 1992 pseudo-cohort (Fig. 5). Immature females (prerecruits) were widely distributed in the

Fig. 4. (*a*) Radiometric shell age (in years) estimated for males in SCI 0 through SCI 5. Circles, *Chionoecetes opilio*; triangles, *Chionoecetes bairdi*. Vertical bars represent measurement error. Horizontal arrows show the approximate mean age of SCI 2 and SCI 3. (*b*) Time series of abundance of females in the south, 1993–1999, by SCI. Circles, SCI 2; squares, SCI 3; triangles, SCI 4*.



Middle Domain of the Central Shelf during the summer of 1991 (Fig. 5*a*), a pattern that is typical for that stage. One year later (summer of 1992), the range of SCI 2 females (necessarily originating from a subset of the 1991 immature pool) had shifted from that of the 1991 immatures in several ways (Fig. 5*b*): (*i*) at the northern end of the Central Domain, the range contracted southwards, (*ii*) in the Middle Domain of the Central Shelf, the range contracted on the east, while on the west, it expanded into adjacent sectors of the Outer Domain, and (*iii*) at the southeastern end of the Middle Domain of the Central Shelf (north of the Pribilof Islands), there was an eastward expansion along the 50-m isobath.

Compared with SCI 2 females in 1992, during the summer of 1993, females in SCI 3 were displaced towards the Outer Domain (Fig. 5*c*), but the direction of the shift varied regionally: (*i*) over much of the Central Shelf, the shift was from northeast to southwest into the Outer Domain, (*ii*) at the southern end of the Central Shelf, west of the Pribilof Islands, the shift was to the south into the Outer Domain, and (*iii*) at the southeastern end, east of the Pribilof Islands, the shift was also southwards from the 50- to the 100-m

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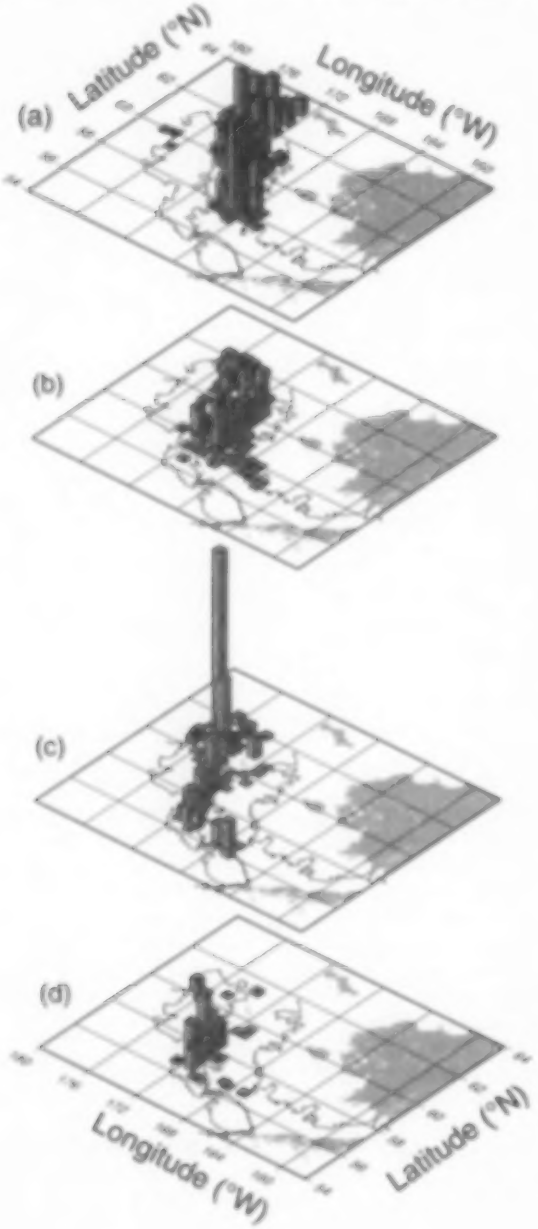

Fig. 5. Snow crab (*Chionoecetes opilio*) pseudo-cohort of 1992. Relative spatial density of life history stages over the eastern Bering Sea. (*a*) Immature females in 1991; (*b*) mature females in SCI 2 (1992); (*c*) mature females in SCI 3 (1993); (*d*) mature females in SCI 4° (1994). Notice the geographic progression of immature and SCI 2 females in the Middle Domain to older stages in the Outer Domain. Isobaths are 50, 100, and 200 m.

isobath, east of the Pribilof Islands. In 1994, the range of the SCI 4° pool (integrated by females of the 1992 pseudo-cohort and older) was generally similar to that of SCI 3 females in 1993, except that it had shrunk southward towards the northern end of the Outer Domain of the Central Shelf (Fig. 5*d*). Contraction of the range could be the result of mortality, movements, or a combination of both; the data do not allow examination of these alternative hypotheses.

To investigate these shifts across pseudo-cohorts, we used the centroids of the geographic range of each pseudo-cohort calculated by SCI (2 and 3). We calculated centroids for the entire region covered by the NMFS surveys and for the northern and southern partitions of the Central Shelf (defined earlier) separately. We focus on the centroids for females in SCI 2 and SCI 3 because (*i*) the average transition time between these is consistently 1 year, (*ii*) most of the mature females sampled in the surveys (86.3 %) were in either SCI 2 or SCI 3, and (*iii*) most of the shift in geographical distribution between SCI groups occurs in the transition between SCI 2 and SCI 3. To track and summarize shifts in the distribution of pseudo-cohorts, we connected the centroids for females in SCI 2 in year i and SCI 3 in year $i + 1$. When the whole region is considered (Fig. 6*a*), all vectors (Table 2) are rooted north of 58°N, reflecting the significance of the northern part of the Middle Domain for recruitment. Most vectors are oriented towards the southwest, the most notable exception being the three earlier pseudo-cohorts followed in the study (1978–1980), which had their recruitment (SCI 2) centroid located north of the Pribilof Islands and shifted to the south–southeast. The mean angle of the vectors (Fig. 6*a*, inset) was 200° clockwise from the north, and the average length of the vectors' moduli was 73.5 nmi (range 37–128 nmi).

Centroids calculated separately for the northern and southern partitions of the Central Shelf are shown in Fig. 6*b*. Vectors in the northern partition point predominantly to the southwest (186–290° clockwise from the north), with a mean angle of 237° and a mean modulus of 74.3 nmi (range 22.9–152.7 nmi). In the southern partition, the pointing direction of the vectors is more variable, but the mean orientation is (as in the northern partition) towards the southwest (mean angle 243° clockwise from the north, mean modulus 54.1 nmi). Whether the Central Shelf is partitioned into northern and southern sectors or not, (*i*) the vectors point (on average) to the southwest of the SCI 2 centroid, (*ii*) the centroids for SCI 2 females are virtually always located in the Middle Domain, and (*iii*) the centroids of SCI 3 females are always located in the Outer Domain or near the border between the Middle and Outer domains.

During the study period, there was a pronounced shift to the north and west of the centroids of geographic distribution of females in SCI 2 and SCI 3 between 1978 and 1985 that continued in a more gradual fashion thereafter (Fig. 7). Between 1978 and 2000, the centroid of SCI 2 females shifted from 58°N in 1978 to north of 60°N in 1998–2000 (120 nmi), mostly between 1978 and 1985. The northward shift of SCI 3 females was steep during the latter period, but the position of the centroid fluctuated around 59°N afterwards. The westward shift (Fig. 7) was sharper in the case of SCI 3 females and (as in the case of the latitudinal shift) occurred mostly between 1978 and 1985.

Environmental gradients

Bottom temperature in the EBS during the summer was investigated using data from the NMFS surveys, which were

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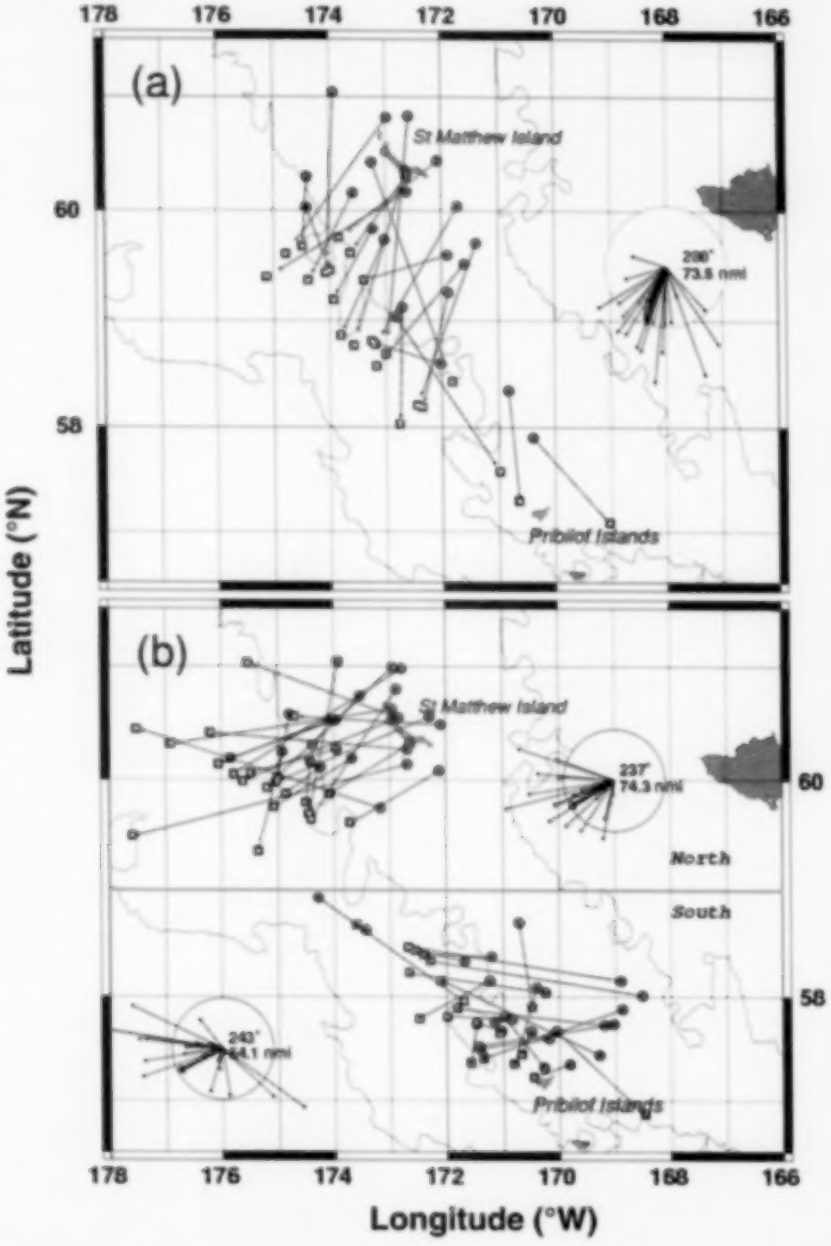
Fig. 6. Centroids of abundance of mature snow crab (*Chionoecetes opilio*) females in SCI 2 (circles) and SCI 3 (squares) by pseudo-cohort. Thin arrows connecting centroids represent average annual migration (distance and azimuth). Inset circles summarize the migration pattern; the thick arrows represent average migration. (*a*) Centroids and migration computed for the entire Central Shelf; (*b*) Central Shelf divided into northern and southern sectors at 59°N.



conducted mostly during late spring – early summer. Although these data provide a good representation of the distribution of bottom temperature over the entire area of interest, there is one important caveat: temperatures were always recorded over a 2- to 3-month period while the surveys progressed from east to west. Thus, there is room for some confounding of within-season temporal trends and spatial pattern.

The typical distribution of NBT during the summer is illustrated for 1996 in Fig. 8*a*. A most significant feature in the context of this study is the cold pool of the Middle Domain. From there, NBT increases towards the Coastal

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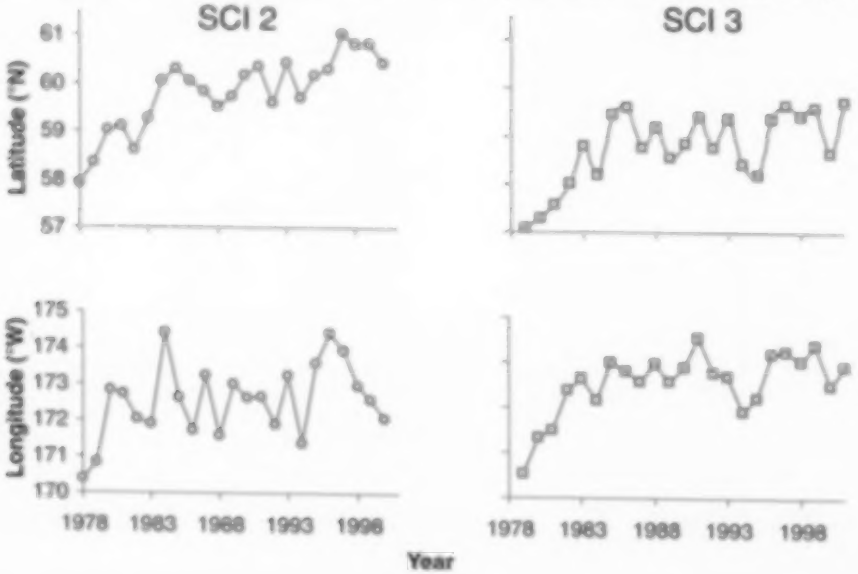
Table 2. Distance between centroids of snow crab (*Chionoecetes opilio*) mature females in SCI 2 during the summer of year *i* and in SCI 3 during the summer of year *i* + 1 (annual pseudo-cohorts 1978–2000).

	Combined		North		South	
	Azimuth	Distance (nmi)	Azimuth	Distance (nmi)	Azimuth	Distance (nmi)
1978	138	65.69	288	137.00	134	70.37
1979	175	63.43	255	152.73	251	21.86
1980	146	104.78	194	54.95	202	14.25
1981	181	65.42	233	22.91	242	45.58
1982	288	40.19	238	49.15	156	7.12
1983	193	65.84	240	55.70	188	22.42
1984	160	36.47	186	22.16	126	99.94
1985	217	50.95	238	51.08	172	48.47
1986	211	87.97	247	88.57	276	84.43
1987	209	44.24	255	47.36	295	99.78
1988	221	74.38	275	104.67	261	78.95
1989	204	58.20	248	63.99	262	39.93
1990	238	88.58	261	118.56	273	36.76
1991	197	98.89	248	88.89	278	77.66
1992	253	47.44	290	83.82	196	45.63
1993	160	128.00	224	36.18	322	37.43
1994	200	96.08	214	82.73	251	84.65
1995	207	53.27	250	59.80	290	14.73
1996	183	37.54	191	34.34	282	28.57
1997	183	96.20	191	81.91	279	120.93
1998	217	90.56	227	91.86	303	5.83
1999	186	129.33	238	104.50	279	121.24
2000	232	66.96	273	76.63	272	38.26
Average	200	73.50	237	74.33	243	54.12

Note: Calculations are presented for the entire Central Shelf and for partitions north and south of 59°N. Azimuth is measured clockwise from the north in degrees and the length of the modulus in nautical miles (nmi) (1 nmi corresponds to a latitudinal minute).

Fig. 7. Time series of centroids of abundance for snow crab (*Chionoecetes opilio*) females in different life history stages.



Domain (to the northeast) and towards the Outer Domain and shelf break (to the southwest). Small patches of relatively warm water surround the Pribilof Islands and to a lesser extent St. Matthew Island. This pattern governs the shape of NBT gradients in the Central Shelf, shown as vector fields for 1996 (Fig. 8*b*) and for the average of 1981–

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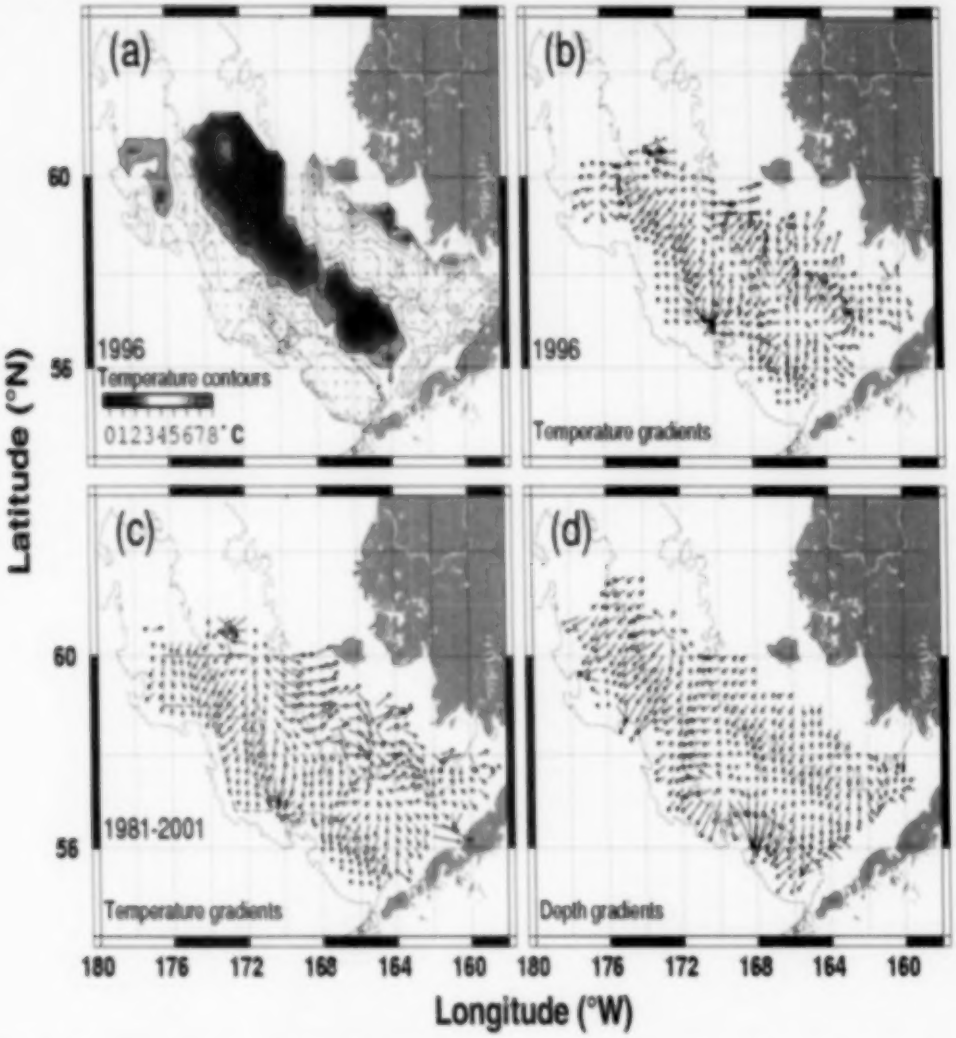
Fig. 8. Summer NBT and depth in the eastern Bering Sea. Points correspond to actual survey stations. (*a*) The 1996 summer NBT contours; (*b*) gradients of NBT during the summer of 1996; (*c*) average gradients of NBT during the summer, period 1981–2001; the polygon shows the station data set used to study environmental variables and migration shown in Fig. 9; (*d*) depth gradients.



2001 (Fig. 8*c*, data for 1975–1980 surveys lack information for many stations). There is a "plateau" (low steepness) region coincidental with the cold pool at the core of the Middle Domain. From there, gradients pointing off the Central Domain (from cold to warm) are grouped into several main vector fields (Figs. 8*b* and 8*c*): (1) towards the Coastal Domain, steep gradients point "downstream" from the west or the northwest, (2) towards the Outer Domain, between St. Matthew and the Pribilof Islands, gradients point to the southwest, (3) in the southeast, east of the Pribilof Islands, gradients pointing to the Outer Domain are oriented to the south, and (4) centripetal gradient fields develop around the Pribilof and St. Matthew islands.

Depth gradients (pointing from shallow to deep) (Fig. 8*d*) are similar to NBT gradients in the case of gradient field 2 but opposite in the case of 1 and 4, as temperature increases towards both the shelf break (deeper) and the coast (including the islands, shallower). As is the case for temperature, steepness of the gradients is minimal over the Central Domain.

Compared with temperature fields (captured by isotherms) (Fig. 8*a*), NBT gradients based on NMFS survey data are likely to be more insensitive to the east–west progression of the survey because the group of neighboring stations used to calculate a vector (typically one central point and its eight nearest neighbors) are generally occupied within a few days. Gradient fields are more conservative than temperature itself, their general configuration being the same during warm and cold years.

Correspondence between environmental gradients and inferred migration route

Let us consider only the region of the Central Shelf where evidence of an ontogenetic migration is stronger, i.e., the

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Middle and Outer domains, north of the Pribilof Islands and west and south of St. Matthew Island (enclosed in a dashed polygon in Fig. 8*c*). There is remarkable correspondence of circular (azimuthal) distributions between (*i*) the proportion of average inferred migration vectors (centroids of SCI 2 in year *i* to SCI 3 in year *i* + 1) and (*ii*) the proportion of average NBT gradients in the Middle Domain quadrant (Fig. 9). There is less matching between either of the two and the circular distribution of depth gradients (which, of course, constitutes a fixed template). In other words, inferred migrations tend to follow the predominant orientation of NBT gradients rather than the fixed topographic gradient template.

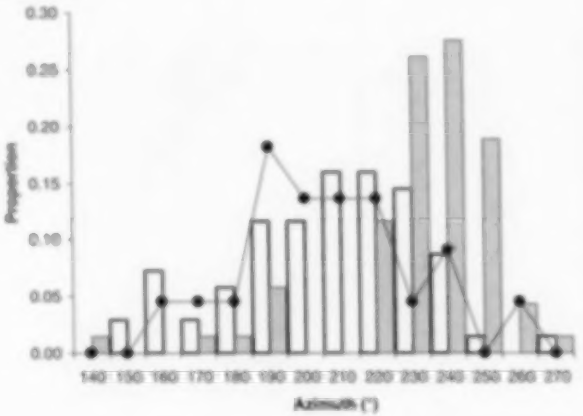
Geographic patterns in the size structure of the mature female population

The existence of a geographical gradient in the average size of mature females in the EBS is shown using the pool of all females sampled in the surveys (Fig. 10). Mature females range in average from more than 70 mm CW at the southeastern end of the range to less than 50 mm CW towards the north, between St. Matthew and St. Lawrence islands. Given that growth stops at the puberty molt (time of recruitment to the mature female pool), geographic patterns in the distribution of average size of pseudo-cohorts should be informative about ontogenetic migration routes. An area where females in SCI 2 are small in year *i* should be connected (through migration) to an area where females in SCI 3 are correspondingly small in year *i* + 1. Provided that there are consistent geographical patterns in the size of mature females, average size could be used as a tracer. The 1988 pseudo-cohort offers a unique opportunity to investigate this hypothesis. In that year, most of the recruits (SCI 2 females) were very concentrated in a large patch located to the southeast of St. Matthew Island (Fig. 11*a*). One year later (summer of 1989), SCI 3 females were concentrated in a dense patch located to the southwest of the 1988 SCI 2 patch (Fig. 11*b*) into the Outer Domain. Average female size contours for the two years (1988–1989) pooled show an intrusion of relatively small females along a corridor that would correspond to the migration path of the 1988 pseudo-cohort (Fig. 11*c*). The corridor has the same orientation as NBT vectors in the same region during the summer of 1988 (Fig. 11*d*).

Discussion

As the members of a snow crab female pseudo-cohort age, there are few ways to track them through time. Under some assumptions (see Materials and methods), radiometric techniques can provide an estimated time since last (in this case terminal) molt. There are several variations of these techniques; radiochemical methods (used by us) take long and are time-consuming. More recently, the use of gamma spectroscopy (a relatively fast technique) has allowed the analysis of large samples (e.g., Gardner et al. 2002), but it requires special facilities (not available to us) and is expensive. In either case, radiometric methods are valuable for calibration purposes but impractical to age large samples. A second method is based on subjective (qualitative) indices of shell deterioration ("condition"), including colonization by epibionts. Among the latter, barnacles can be aged, providing a

Fig. 9. Distribution of the orientation of depth gradients (shaded bars), temperature gradients (open bars), and ontogenetic migration (SCI 2 to SCI 3) around the azimuth (in degrees with respect to true north) (circles). The frequency of temperature and depth gradients was computed for the area indicated in Figs. 8*c* and 8*d*, which overlaps most with inferred routes of ontogenetic migration.



lower boundary estimate of the age of the host's shell (e.g., Paul and Paul 1986). We disregarded this alternative because of the fact that barnacles were present on only 20% of the females processed in the laboratory. We used the radiometric analyses to provide a calibration and also attempted tracking identifiable pseudo-cohorts through time. The results of the two methods coincide: SCI 2 and SCI 3 largely correspond to, respectively, females that molted during the current calendar year and during the previous one. Thus, SCI 2 females are the primiparous recruits to the female pool. In the case of an annual reproductive cycle, SCI 3 females would be first-time multiparous.

The life history schedule of mature females in the EBS is poorly known. The timing of the puberty molt has not been documented in the EBS, although it is over by the time the annual NMFS survey is conducted (May–August). Our radiometric results indicate that the female terminal molt (and consequently primiparous mating) in the EBS must occur during the winter (February–March). Interestingly, this is also the case for eastern Canada, where it occurs in February–April, beginning before the ice breakup (Moriyasu et al. 1987; Sainte-Marie 1993; Sainte-Marie et al. 1995 and references therein). In the EBS, hatching and subsequent oviposition start in March and are completed by July (Somerton 1981*b*). This, too, is comparable with eastern Canada, where hatching of mature eggs, mating, and subsequent extrusion of new eggs occur in April-June (Taylor et al. 1985; Sainte-Marie 1993; Comeau et al. 1999). Thus, as is also the case in eastern Canada, primiparous mating takes place 2–3 months earlier than multiparous mating. For the same reason, primiparous females carry eggs for a longer period than multiparous females (approximately 14 versus 12 months in the case of an annual reproductive cycle).

In eastern Canada, the timing of the terminal molt of males has been observed in aquaria in winter and early spring (February – late April; Conan et al. 1990) and was inferred

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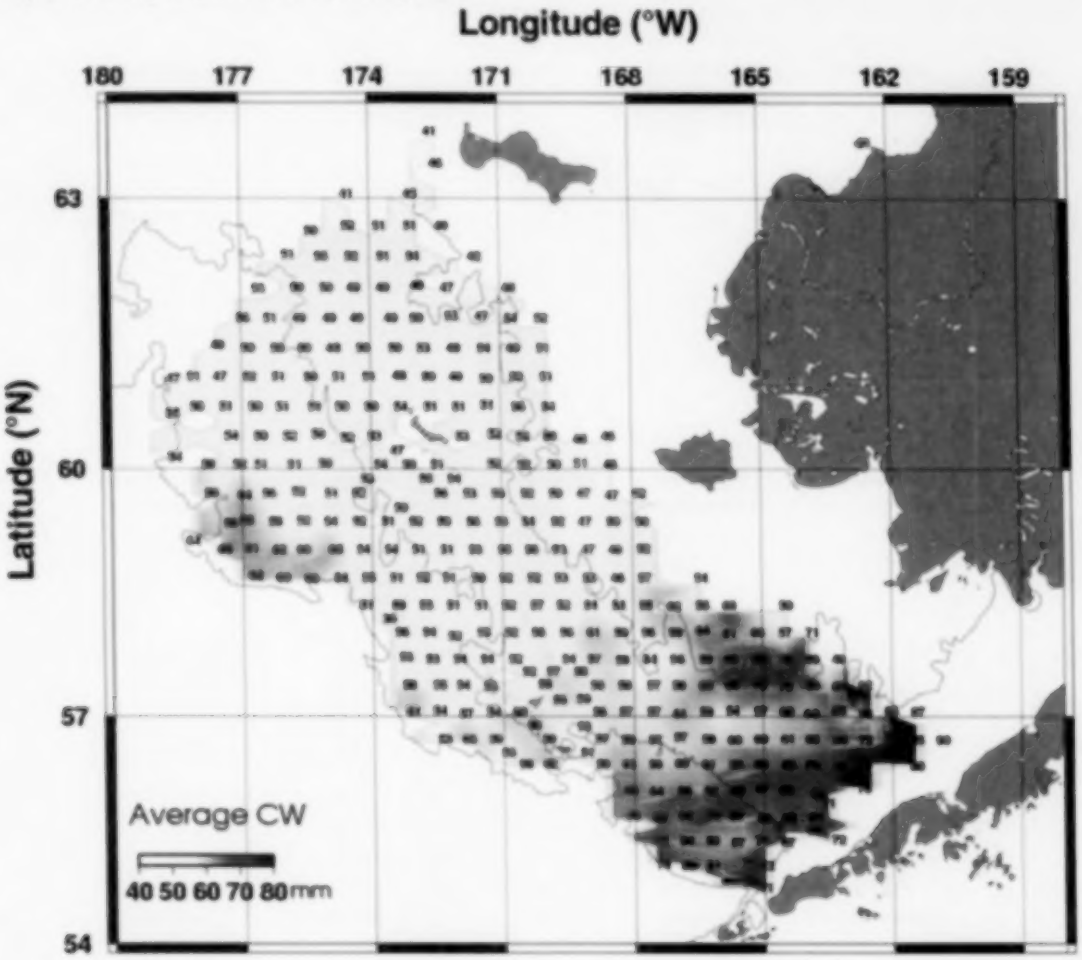
Fig. 10. Average CW of mature snow crab (*Chionoecetes opilio*) females, all SCIs and 1978–2001 surveys combined. Individual numbers correspond to average CW at each NMFS survey station.



to peak in the field during the spring (March–May) (Sainte-Marie et al. 1995 and references therein; Conan et al. 1996). Consequently, the puberty molt of females peaks before the terminal molt of males, possibly allowing the latter to mate with primiparous females before molting into morphological maturity. The timing appears to be the same in the EBS (B. Ernst, J.M. Orensanz, and D.A. Armstrong, unpublished data), as the proportion of recently molted (SCI 1) large males (CW > 102 mm, presumably morphologically mature) caught by the NMFS surveys (aggregated data) peaks in June and July (23.8% and 21.9%, respectively, of the large males). According to our radiometric results, those SCI 1 males should have molted up to 3 months earlier. We hypothesize that the pseudo-cohorts of morphologically mature males that were seen as recently molted (SCI 1) during the summer surveys are likely to migrate offshore, becoming available to the fishery in the Outer Domain during the next winter as "clean-shell" crabs. A very similar phenomenon has been proposed for eastern Canada populations. After males and females molt into maturity in shallow water during late winter and spring, a radiotelemetry study showed that while females remained in shallow water, males moved offshore (Conan et al. 1996). If disturbed, morphologically mature males can move several miles in a few days. These observations are consistent with the hypothesis that recruitment of large males to deep coastal fishing grounds occurs following a down-slope movement of recently molted males (Coulombe et al. 1985; Bouchard et al. 1986; Sainte-Marie and Hazel 1992).

Somerton (1981*b*) was the first to track a pseudo-cohort of snow crab mature females in the EBS. He tracked the 1969 pseudo-cohort through the early 1970s and concluded that maximum life after terminal molt is approximately 5 years. Both radiometric methods (Nevisi et al. 1996; this study) and tracking of an identifiable pseudo-cohort (this study) suggest that maximum post-terminal molt life span in the EBS is on the order of 6–7 years. This, again, is in line with estimates from eastern Canada, where it has been estimated by means of tagging (Sainte Marie et al. 1996) and radiometry (Conan et al. 1996). These studies indicate that it

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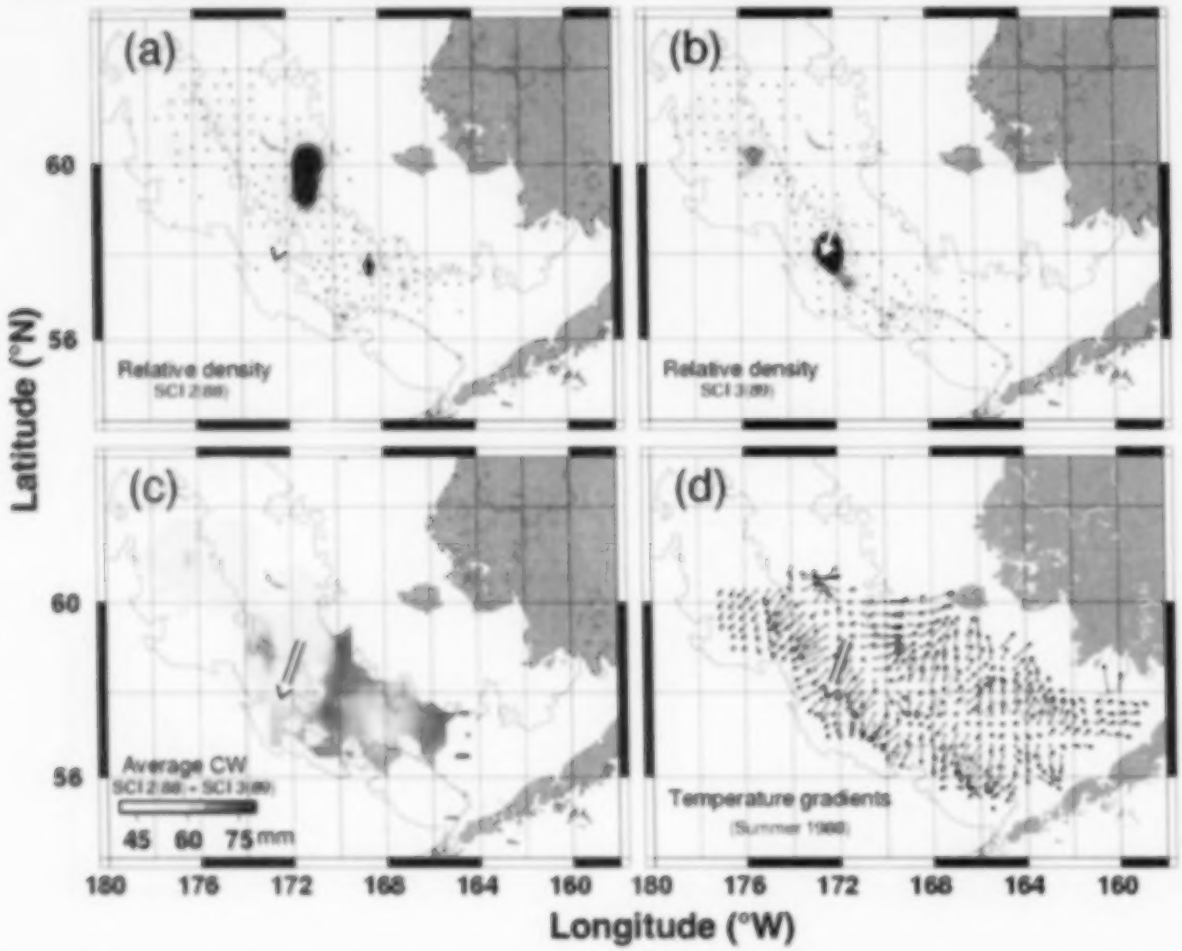
Fig. 11. Tracking of the 1988 snow crab (*Chionoecetes opilio*) pseudo-cohort. Dots correspond to NMFS survey stations with recorded abundance. The large arrow indicates the direction of movement of the core of the pseudo-cohort distribution. (*a*) Abundance of SCI 2 females in 1988; (*b*) abundance of SCI 3 females in 1989; (*c*) surface plot of CW of individuals of SCI 2 in 1988 and SCI 3 in 1989 combined; (*d*) NBT gradients during the summer of 1988.



can reach a maximum of 5–6 years. We conclude that the annual schedule of life history events from the terminal molt onwards is virtually the same in eastern Canada and the EBS for both females and males.

While differential patterns of distribution of snow crab life history stages in the EBS have long been recognized (Somerton 1981*b*), it has been traditionally held that migrations in the EBS are negligible, if present at all (Somerton 1981*a*; Incze et al. 1987). However, there is growing evidence of an extensive ontogenetic migration of both males and females from shallower to deeper waters (Otto 1998), with a predominant northeast–southwest direction (Orensanz and Armstrong 1997; Zheng et al. 2001). This can be inferred from the spatial shifts in the distribution of SCI categories, whether cohorts are pooled or not. Tagging experiments conducted in the EBS between 1978 and 1982 showed an average displacement of 75 km for males (McBride 1982).

In this study, we expanded from those preliminary indications. Females become vulnerable to the NMFS survey gear when they reach a size range (30–60 mm CW) that is roughly equivalent to the three instars preceding the terminal molt, presumably owing to size selectivity of the trawl gear. This pool of immature females is concentrated in the Middle Domain and adjacent fringe of the Coastal Domain of the Central Shelf. For smaller crabs, the only piece of information available on a regional scale comes from fish stomach contents, predominantly Pacific cod (*Gadus macrocephalus*) caught during the same NMFS surveys (Livingston 1989). Livingston (1989) found that snow crabs (size range 3–87 mm but predominantly smaller than 35 mm, her fig. 6) tend to occur in stomachs of Pacific cod caught in the Middle Domain or adjacent sectors of the Coastal Domain (her fig. 3). This is virtually the same geographical range observed for immature females caught in the surveys.

Female recruits to the mature population (SCI 2) are also concentrated in the Middle Domain of the Central Shelf but slightly displaced towards the Outer Domain in comparison with immatures. Females in SCI 3 (1 year past terminal

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molt) appear displaced towards the Outer Domain, a pattern that emerges both in the aggregated data and when individual pseudo-cohorts are tracked (e.g., the 1992 pseudo-cohort). The consistency and recurrence of the pattern could not be possibly explained by mechanisms other than an ontogenetic migration (e.g., differential survival). Errors in the assignation of individuals to SCI categories may affect the estimated rate of annual migration but not the basic pattern or the orientation of the migration. Assignation errors would result in an underestimation of the annual rate of migration, as centroids for the two groups would be pulled closer to each other. Older females (SCI 4+ pool) are concentrated in the Outer Domain. The transition between SCI 2 and SCI 3 is the most significant in terms of the average distance of the migration (approximately 75 nmi) and the reproductive contribution of the stages involved. The reproductive contribution of older females (SCI 4+ pool) is likely to be of limited significance owing to mortality (only 13.7% of the mature females caught over 25 years were in that group) and reduced fecundity related to senescence.

Given that females stop growing after terminal molt and that there is variation in size at maturity between pseudo-cohorts, strong pulses composed of exceptionally large or small individuals could be tracked in time and space, as they contrast with the background population (pool of other pseudo-cohorts). In the case of males, this edge was utilized by Otto (1998), who was able to track a strong pseudo-cohort of morphologically mature males, identifiable because of its small size, as it vanished from shallow areas (<80 m) after 1990, showing up in deeper water in 1991. In a similar way, we were able to track a strong, localized pulse of small-sized recruits of the 1988 pseudo-cohort as they intruded (with a southwest direction) across a background region characterized by larger females.

An ontogenetic unidirectional offshore migration has been also reported for shelf/slope populations of *Chionoecetes tanneri* (Pereyra 1966) and snow crabs of Japan (Ito 1968; Kon 1969; Yamasaki and Kuwahara 1993). In the latter, females molt to maturity and mate at a depth of 200–300 m. Males and females migrate offshore afterwards, the male migration being far more reaching; males, on average, end up in deeper areas than females, apparently not returning to shallower areas.

The ontogenetic migration of female snow crabs in the EBS, as seen through the "window" of NMFS' summer surveys, appears to be unidirectional. We hypothesize that during their directed ontogenetic migration, female snow crabs track environmental gradients. Two possibilities are depth and NBT. These variables are often illustrated in the form of isolines (isobaths and isotherms), but the resulting charts are of limited value in the interpretation of crab movements, as the latter do not "see" a map integrating information for a large region but, rather, respond to conditions in their immediate vicinity. Considering their sensorial complement, crabs are more likely to follow temperature than depth gradients, although the latter could be correlated with other factors, such as sediment composition or temperature itself. Experiments conducted by Dionne et al. (2003) demonstrated that juvenile snow crabs make active choices based on water temperature and substrate. Starting from the Middle Domain (low temperature and intermediate depth) where the offshore ontogenetic migration of mature females begins, summer NBT and depth gradients do not run in the same direction offshore (towards the Outer Domain, deeper and warmer water) and onshore (towards the Coastal Domain, shallower and warmer water). Considering summer gradients only, and given that there is no evidence of an inshore migration into the Coastal Domain, the most parsimonious assumption would be that females track the depth gradient, which would consistently lead them offshore. During the winter, however, as the ice cover develops, water in the Coastal Domain becomes colder and the gradient, although not well documented for that time of the year, reverses its direction (Phyllis Stabeno, National Marine Fisheries Service, 7600 Sand Point Way Northeast, Seattle, WA 98115, USA, personal communication). From the Middle Domain towards the Outer Domain, the gradient of NBT is colder to warmer year-round. Orientation of the integrated annual migration of primiparous females (SCI 2 females in year i to SCI 3 females in year $i + 1$) is remarkably similar to summer NBT gradients observed between the Middle and Outer domains. The distribution of migration vectors matches the distribution of NBT gradients better than it does the depth gradient template. We hypothesise that snow crabs in the EBS generally track NBT gradients and that onshore movements away from the Middle Domain are prevented by the winter cooling and excessive summer warming of the Coastal Domain. Alternatively, it could be hypothesized that mature primiparous females move onshore during the fall and return during the spring. This seasonal migratory cycle is unlikely given that SCI 3 females (the presumable condition of females migrating back to the Middle Domain during the spring) are virtually absent during the summer from fringe areas of the Middle Domain adjacent to the Coastal Domain.

A relationship between snow crab distribution and temperature has been documented for the EBS (Somerton 1981*b*), the Sea of Japan (Yosho and Hayashi 1994), and eastern Canada (Tremblay 1997). Temperature-related shifts in spatial distribution have been documented for eastern Canada. Dionne et al. (2003) argued that early benthic stages of snow crab (instars I–IV) are stenothermic, occurring in the northwest Gulf of St. Lawrence immediately above and below the core of the cold intermediate layer at NBT > 0 °C. Consistently, the thermopreferendum observed in laboratory experiments was in the range 0–1.5 °C. The thermopreferendum shifted in the laboratory to a warmer range (1.0–4.5 °C) at instars III and V (Dionne et al. 2003). A similar relationship could exist in the EBS with the cold pool (NBT < 2 °C) that develops over the Middle Domain during the spring and summer (Somerton 1981*b*; Livingston 1989).

Seasonal migrations between the Outer and Middle domains of the EBS could not be investigated because surveys are conducted only during the summer. Although an eventual offshore–onshore seasonal component superimposed to the offshore ontogenetic migration of females cannot be captured through the "windows" available (the NMFS summer survey), it seems to exist in males (B. Ernst and J.M. Orensanz, unpublished data). If seasonal migrations did exist, then seasonal female "onshore" (to shallower water) net displacements should be shorter than the subsequent "offshore" displacements. The result, as observed through the summer window, would still be a unidirectional ontogenetic migra-

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tion. A phenomenon of this type was documented by Bréthes and Coulombe (1990) for males off the north shore of the Gulf of St. Lawrence. Snow crab seasonal offshore–onshore migrations have been well documented in the Gulf of St. Lawrence ecosystem (eastern Canada), where male and female crabs are present in shallow waters during late winter and early spring (Taylor et al. 1985; Sainte-Marie and Hazel 1992; Conan et al. 1996). Some adult males move into the shallow waters in December (coincident with the start of the pubescent female molting period), while the abundance of adolescent males increases over the winter, suggesting a slightly later arrival (Lovrich et al. 1995). The conditions that elicit seasonal aggregation in sallow-water locations are unknown; they have been variously interpreted as the result of a directed reproductive migration or, more generally, tracking temperature gradients following stage-specific thermopreferenda (Sainte-Marie and Hazel 1992). Crabs move away from the shallow coastal area during the summer, when surface temperature begins to warm and exceeds the core temperature of the cold intermediate layer.

Our hypothesis about the ontogenetic migration being driven by NBT implies the assumption of no upper limit to thermal preference (at least within the geographic domain of interest). Seasonal reversals in the prevalent direction of migration would require a more complex hypothesis, including ontogenetic changes in the thermopreferendum (e.g., as in Dionne et al. 2003) and (or) the nonlinearity of thermal preference.

A full comparative discussion with eastern Canadian scenarios is not possible because environmental variables are unevenly documented and have not been analyzed in the same way in the different systems. It is likely, however, that while the timing of life history events appears to be very similar between eastern Canada and the EBS, this may not apply to patterns of migration. Resultant directionality of the ontogenetic migration and onshore–offshore seasonality may be different in the two systems owing to the different ways in which NBT gradients are structured, in particular relative to the cold intermediate layer in the Gulf of St. Lawrence and the cold pool in the EBS. In the EBS, there is no indication of a seasonal migration into the Coastal Domain, which would be analogous to seasonal migrations to shallow waters in the Gulf of St. Lawrence (Lovrich et al. 1995). Ontogenetic migration is directed, instead, towards the deeper and warmer waters of the Outer Domain. Migration towards deeper waters for access to warm water is not a viable option for juvenile snow crabs in the Gulf of St. Lawrence, considering the severity of hypoxia at depth (Dionne et al. 2003).

The ecosystem in which snow crabs evolved is unknown and so the original adaptive significance of behavioral characters observed in extant populations cannot be interpreted with certainty to provide an explanation for movements in the EBS. The various systems that now harbor snow crab populations on both sides of North America are relatively recent, as all originated after the retreat of ice during the last postglacial period. It is not likely that in such a brief time, local populations were fine-tuned by natural selection in characters such as the nature of the environmental gradients tracked during migrations. More likely, snow crabs may prosper in different systems with the same basic repertoire of relatively simple behavioral responses, a possible result being that crabs end up doing different things at different locations, yet for the same reasons.

Whichever the case, we show that the female population of snow crabs of the EBS has strong spatial structure and a directed ontogenetic migration, but the latter cannot be invoked to justify homogeneity assumptions. At a reasonably large scale (onthe order of kilometres), a perturbation of the mating system at a given location (e.g., removal of males by the fishery) may be compensated by down-gradient migration in one direction (e.g., northeast–southwest) but not by cross-gradient displacements in the orthogonal direction (e.g., northwest–southeast).

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Nonlethal estimation of proximate composition in fish

M. Keith Cox and Kyle J. Hartman

Abstract: The need to precisely measure growth is a common denominator in many fisheries studies, but growth measures other than total masses or lengths are nearly nonexistent because more precise measurements such as body composition analysis are often too difficult and time consuming. Here, we present a means of estimating body composition in fish quickly, and after validation, without the need to sacrifice the animal. Models built with brook trout (*Salvelinus fontinalis*) were linear with strong validation group relationships ($R^2 > 0.96$) for composition parameters including water, protein, fat, fat-free, and dry masses. Subject responses to bioelectrical impedance analysis were minimal, with only slight bruising ($p < 0.001$) with no effect on swimming, color, bleeding, or feeding. The model was also tested on the water and dry masses of 10 warmwater fish species and found to have strong correlations ($R^2 > 0.86$), suggesting that more general relationships may exist. Nonlethal estimation of body composition using bioelectrical impedance analysis will permit increased precision in bioenergetics energy flow and compositional studies as well as permit study of community energetics and condition on spatial and temporal scales not previously possible.

Résumé : Une mesure précise de la croissance est nécessaire dans plusieurs études sur les poissons, mais il n'existe à peu près pas de variables mesurées autres que les masses ou les longueurs totales, parce que les variables plus précises, telles que la composition corporelle, sont souvent trop difficiles ou trop onéreuses à analyser. Nous proposons une méthode rapide de détermination de la composition corporelle des poissons qui, une fois validée, ne nécessite pas le sacrifice de l'animal. Les modèles élaborés pour l'omble de fontaine (*Salvelinus fontinalis*) sont linéaires et possèdent de fortes relations de validation de groupe ($R^2 > 0{,}96$) pour les variables de la composition, telles que l'eau, les protéines, les graisses, la masse sans les graisses et la masse sèche. La réaction individuelle à l'analyse par impédance bioélectrique est minime et consiste en de légères ecchymoses ($p < 0{,}001$) sans effet sur la nage, la coloration, le saignement et l'alimentation. Le modèle a aussi été utilisé pour déterminer la masse hydrique et la masse sèche de 10 espèces de poissons d'eau chaude; les corrélations sont fortes ($R^2 > 0{,}86$), ce qui laisse croire qu'il peut exister des relations plus générales. L'estimation sans sacrifice du poisson de la composition corporelle par l'anayse par impédance bioélectrique permet une plus grande précision dans la détermination des flux dans les études bioénergétiques et dans les analyses de composition corporelle; elle permet aussi l'étude de la bioénergétique des communautés et de la condition physique à des échelles spatiales et temporelles impossibles à envisager jusqu'à maintenant.

[Traduit par la Rédaction]

Introduction

Growth is considered the ultimate expression of well being at the individual level and is also linked to the reproductive success of an organism (Shine and Schwarqkopf 1992; Brandt and Hartman 1993; Elliott and Hurley 1995). Actual growth reflects changes in the body composition or mass of growth components (e.g., fat and protein) relative to inert (ash) or compensating (water) components. However, the definition of growth in most studies has been limited by the ability to accurately measure energetic growth. Since most animals are between 60% and 90% water, changes in this compartment can greatly influence estimates of growth based on total mass. In most growth studies, the more precise measurements of body composition have often been shelved because a quick, reliable, inexpensive method to estimate body composition is lacking.

A variety of in vivo and in vitro methods are available for assessing proximate composition in organisms, but most appear to be impractical, inconvenient, or cost-prohibitive for field or ecological type work (Lukaski 1987). Proximate analysis of compartments includes total body water (TBW), dry mass (DM), fat-free mass (FFM), total body protein (TBP), total body ash (TBA), and total body fat mass (TBF). Direct measurements of individual compartments using chemical analysis require sacrificing the organism followed by lengthy laboratory procedures (Association of Official Analytical Chemists 1990). This negates the ability for repeated measures on the same individual and suppresses compositional study of endangered species. Certain compo-

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nents such as TBW are good predictors of other body composition estimations, but in vivo methods to estimate TBW involve using radioisotope tracers such as tritium, deuterium, and oxygen-18, which are difficult to analyze outside a specialized laboratory and are also cost-prohibitive (Lukaski 1987). FFM can be estimated nonsacrificially by counting intracellular potassium-40 with thallium-activated sodium iodide crystal detectors, but the high cost, instrumentation, and technicality diminish its practicality (Corcoran et al. 2000).

Electrical conductivity methods are nonsacrificial and include total body electrical conductivity (TOBEC) and bioelectrical impedance analysis (BIA), which both rely on impedance differences between fat and fat-free tissues. Lantry et al. (1999) found that the TOBEC method accurately estimates composition parameters for healthy individuals, but errors increase when subjects are undergoing mass or compositional changes or when subjects with dissimilar body sizes are compared (conductivity changes with body geometry and size). Furthermore, the TOBEC unit is large and nonportable, making field studies impractical. BIA is available in an inexpensive, small, portable unit that has provided accurate and reliable estimations of water mass in humans, but as with the TOBEC, complex body geometries tend to decrease the accuracy of the composition estimations. However, we hypothesize that if BIA technology is applied to organisms with a more simplified body geometry (majority of the mass located in the a single volume), accuracy of measurements would not be lost and proximate composition estimations could accurately and nonlethally be made in laboratory and field settings for fish. Furthermore, because of the generality of electrical resistivity laws across various mediums, models for fish would not seem to have the restrictions of individual species specificity.

To investigate the applicability of BIA technology on fishes, we measured brook trout (*Salvelinus fontinalis*) for bioelectrical impedance and sacrificed for analysis of total body proximate composition components. Independent predictive models were made for TBW, DW, FFM, TBP, TBA, and TBF using derivatives of resistance and reactance equations. These models were then tested on an independent validation group of brook trout. Values from each predicted composition parameter were compared with actual numbers obtained from laboratory proximate analysis. Generality of the brook trout model was tested on a second validation group that consisted of 10 species of warmwater fishes. Predicted values of DW, TBW, and total mass were compared with actual values. An additional group of brook trout was used to test for harmful responses to BIA methods.

Materials and methods

Principles of BIA

Proximate composition estimations are calculated by measuring the impedance (resistance and reactance) of a current (800 μA AC and 50 kHz) passed through an organism and then regressing these measures with actual proximate body composition numbers for that organism. Resistance of a substance is proportional to the voltage of an applied current as it passes through a substance, or

$$(1) \quad R = V \cdot CT^{-1}$$

where R is resistance (ohms), V is applied voltage (volts), and CT is current (amps). Reactance X_c (ohms) is the opposition to alternating current by a capacitor (cell membranes) and can be mathematically expressed by the following equation:

$$(2) \quad X_c = 1 \cdot 2\pi f C^{-1}$$

where f is frequency (hertz) and C is capacitance (farads) (Keller et al. 1993). Impedance (R and (σ) X_c) is related to the cross-sectional area, conductor length of the organism, and the signal frequency of the current (Lukaski 1987). Impedance can be expressed by the equation

$$(3) \quad Z = \rho L \cdot A^{-1}$$

where Z is impedance (ohms), ρ is a resistivity constant, L is measured length, and A is cross-sectional area (square centimetres). If the signal frequency and the configuration are held constant, the impedance measurements can be related to its volume. This is demonstrated by the following: if both sides of the equation are multiplied by L/L, then

$$(4) \quad Z = \rho L^2 L \cdot A^{-1}$$

Resistance represents conductive material more than X_c so Z can be represented by R

$$(5) \quad R = \rho L^2 L \cdot A^{-1}$$

where $L \cdot A$ represents a volume (cubic centimetres), and by substitution

$$(6) \quad V_\varepsilon = \rho L^2 \cdot R^{-1}$$

where V_ε is volume epsilon (cubic millimetres) and ρ is determined statistically by regressions of V_ε on impedance. Cell membranes consist of a nonconductive lipid bilayer sandwiched between two conductive protein layers, and at the low voltages and high frequencies such as those used here, current mainly passes through extracellular fluids, while at higher frequencies, the cell walls become capacitive (Pethig 1979). This allows reactance and resistance numbers to be sensitive to changes in volume of extracellular and cellular material.

Model development and validation

A total of 50 brook trout, 22 from headwater streams and 28 from the Bowden State fish hatchery, Bowden, West Virginia, were placed into a data set from which the model and validation groups were selected (Table 1). The model group consisted of 30 fish that (*i*) satisfied conditions of having individual fish with the maximum and minimum values for length (110–285 mm), mass (10–241 g), resistance (389–786 Ω), and reactance (59–213 Ω) and (*ii*) were randomly selected until a total number of 30 had been reached. The remaining 20 fish not placed in the model group were placed in the validation group. It was important to include relatively fat and lean fish in the model to provide sufficient variation in fat masses to allow us to adequately evaluate the ability of BIA to predict this parameter. We assumed that the hatchery fish were in a fat condition owing to their regular, three times daily ad libitum feeding schedule. Therefore, BIA pro-

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Table 1. Mean values for length, mass, fat mass, and impedance values for all species in this study.

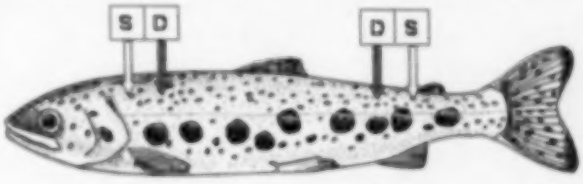
Species	*n*	Length (mm)	Mass (g)	Fat mass (g)	% fat (range)	Resistance (Ω)	Reactance (Ω)
Brook trout (*Salvelinus fontinalis*)	30	165.44 (53.25)	60.77 (67.54)	3.75 (5.88)	0.67–9.55	570.60 (115.75)	160.73 (34.18)
Validation	20	177.55 (51.16)	68.84 (64.51)	4.00 (6.04)	1.50–9.28	532.05 (118.16)	150.20 (26.40)
Freshwater drum (*Aplodinotus grunniens*)	11	236.45 (42.14)	140.82 (82.05)	na		431.91 (30.39)	132.09 (15.58)
Gizzard shad (*Dorosoma cepedianum*)	15	212.20 (30.24)	73.11 (37.16)	na		473.13 (73.30)	168.53 (43.42)
Longear sunfish (*Lepomis megalotis*)	10	124.90 (14.37)	45.47 (18.57)	na		318.10 (67.93)	91.30 (11.83)
Logperch (*Percina caprodes*)	6	115.67 (7.89)	11.39 (2.42)	na		509.00 (92.16)	163.33 (18.91)
Moxostoma spp.	11	186.55 (69.13)	97.55 (123.21)	na		392.27 (48.48)	118.45 (15.85)
Redbreast sunfish (*Lepomis auritus*)	4	126.00 (35.99)	44.71 (28.87)	na		329.75 (41.76)	69.75 (10.50)
Sauger (*Sander canadensis*)	11	273.55 (32.79)	156.50 (61.69)	na		386.36 (58.53)	107.00 (17.68)
Smallmouth bass (*Micropterus dolomieu*)	11	176.00 (36.62)	66.10 (38.67)	na		370.09 (34.63)	124.09 (32.11)

Note: Here, brook trout (*Salvelinus fontinalis*) refers to the fish used in the brook trout model and validation refers to fish used in the validation set for the brook trout model. Values in parentheses represent 1 SD. na, not applicable.

cedures (below) were performed on 10 hatchery fish on site (representing relatively fat fish) and the remaining 18 fish were transported back to the West Virginia University Ecophysiology Laboratory. Transported fish were placed and maintained in recirculating tanks (0.5 m × 1.5 m) at 14 ± 1 °C and withheld from food for 30 days to achieve lower fat levels. The 22 stream fish were captured by electrofishing and analyzed with BIA (following the procedures below). Gender was not determined in fish.

Fish in both the model and validation groups were anesthetized and measured for BIA in an identical manner. Fish were anesthetized in a tricaine methanesulfonate solution of 1 g/9 L water, blot dried, and placed on a nonconductive board in a left lateral recumbent position. Electrical impedance (resistance and reactance) was measured with a tetrapolar bioelectrical impedance analyzer (RJL Systems, Detroit, Michigan). The analyzer has two sets of needle electrodes (stainless, 28 gauge, 12 mm) (Grass Telefactor, West Warwick, Rhode Island) with each set consisting of one signal and one detecting electrode placed 1.0 cm apart. One set was placed in the anteriad dorsad region and the second set was placed in the caudal peduncle region of the fish (Fig. 1). Each set of electrodes was placed in a consistent position for each fish: midway between the lateral line and dorsal midpoint with the anterior set of electrodes positioned at the anterior apex of the operculum and the posterior set positioned even with the anterior edge of the adipose fin (Fig. 1). Electrode needles penetrated 2 mm into the fish. The distance between the two detecting electrodes was measured for each fish, and a current was introduced through the signal electrodes and the proximal detecting electrodes measured the voltage drop. These two electrical values, resistance and reactance, were then used to calculate values from common electrical property equations that included resistance in series and in parallel, reactance in series and in parallel, combined resistance and reactance in series and in parallel, and capacitance. These values were then used as independent variables in the regression model.

Fig. 1. Diagram of locations for signal (S) and detector (D) electrodes used in measuring bioelectrical impedance values of brook trout (*Salvelinus fontinalis*). Brook trout drawing modified from the Oregon Department of Fish and Wildlife, Salem, Oregon.



Dependent variables in the regression model were the body composition values measured in the laboratory immediately following BIA procedures. While still anesthetized, fish were measured for length and mass and euthanatized with an overdose of tricaine methanesulfonate (solution of 3 g/9 L water) and frozen. The individual parameters and methods used to quantify each were analyzed by the West Virginia University Rumen Profiling Fermentation Laboratory and are as follows: TBP by the Kjalkahl method, TBA in an ashing oven at 500 °C, TBW by back-calculating dried samples, DM by oven drying at 100 °C, TBF by ether extraction, and FFM by mass balance equations (body mass minus TBF) (Association of Official Analytical Chemists 1990). Independent models for each parameter were built from the model group. Relationships between the actual and predicted values were explained with correlation analysis for strength of linear relationships and predictability, residual plots and *F* values (to test significance of slopes in linear regressions), and confidence limits on the regression coefficients (to test for 1:1 relationships).

Best-fit linear models were then used to predict parameter estimations in both the brook trout and warmwater species validation groups. Prediction values for each parameter in both validation groups were expressed by the following equation:

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Table 2. Relationships between proximate body composition components and impedance equations for brook trout (*Salvelinus fontinalis*) in the model group.

Component	*n*	Intercept (SE)	Slope (SE)	E	*F* statistic (*p* value)	R^2
TBW	30	1.320 45 (1.948 26)	3.461 87 (0.105 60)	L^2/R_m	<0.0001	0.9746
DM	30	0.295 58 (0.778 43)	4.315 75 (0.136 79)	L^2/X_{cp}	<0.0001	0.9726
FFM	30	−0.518 81 (0.561 08)	0.999 68 (0.030 41)	L^2/R_m	<0.0001	0.9747
TBP	30	−0.673 52 (0.523 28)	0.896 30 (0.028 36)	L^2/R_m	<0.0001	0.9727
TBA	30	0.115 16 (0.060 89)	0.117 49 (0.003 70)	L^2/R_p	<0.0001	0.9730
TBF	30	−1.790 87 (0.320 15)	$1.803\,82^{-23}$ ($7.289\,85^{-25}$)	L^2/X_{cp}	<0.0001	0.9563

Note: Analysis results of the linear relationships between the measured bioelectrical impedance equations and actual numbers of proximate body composition components: total body water (TBW), dry mass (DM), fat-free mass (FFM), total body protein (TBP), total body ash (TBA), and total body fat mass (TBF). In the table, "E" represents the specific impedance equation providing the best fit, where L is length, R is resistance, Z is impedance, X_c is reactance, and subscripts "p" and "m" represent parallel and series circuitry, respectively.

Table 3. Correlation coefficient scores (r) of predicted and actual parameter values including dry mass (DM), total body water (TBW), and total mass (summation of DM and TBW versus actual wet mass of fish) for various warmwater species using the brook trout (*Salvelinus fontinalis*) bioelectrical impedance analysis (BIA) model.

Species	*n*	*r*		
		DM	TBW	Total mass
Freshwater drum (*Aplodinotus grunniens*)	11	0.992 06	0.999 76	0.999 74
Gizzard shad (*Dorosoma cepedianum*)	15	0.992 08	0.999 12	0.998 18
Longear sunfish (*Lepomis megalotis*)	10	0.861 70	0.998 63	0.996 08
Logperch (*Percina caprodes*)	6	0.878 16	0.995 29	0.990 83
Moxostoma spp.	11	0.997 91	0.999 45	0.999 81
Redbreast sunfish (*Lepomis auritus*)	4	0.858 39	0.999 41	0.996 69
Sauger (*Sander canadensis*)	11	0.942 27	0.999 69	0.996 02
Smallmouth bass (*Micropterus dolomieu*)	11	0.907 06	0.999 81	0.994 52

Note: Spotted bass (*Micropterus punctulatus*) and white crappie (*Pomoxis annularis*) were not included here because of low sample sizes (one fish each).

$$(7) \qquad PP = (PP/PM) \times \text{mass}$$

where *PP* is the new predicted parameter value (grams), PP is the initial predicted parameter (grams), PM is the predicted total mass (grams), and mass is the measured actual wet mass of the fish. Statistical and analytical procedures were completed with SAS® 8.1 software. The best-fit linear models were then tested using the validation group.

Generality of the brook trout model

To test brook trout model generality, water and DM models developed for brook trout were used to predict TBW and DM of a group of warmwater fish. Eighty-one fish consisting of 10 species were collected in the summer of 2003 by nighttime electroshocking on the Kanawha River in Charleston, West Virginia. Species included white crappie (*Pomoxis annularis*), freshwater drum (*Aplodinotus grunniens*), gizzard shad (*Dorsoma cepedianum*), longear sunfish (*Lepomis megalotis*), logperch (*Percina caprodes*), *Moxostoma* spp., redbreast sunfish (*Lepomis auritus*), sauger (*Sander canadensis*), smallmouth bass (*Micropterus dolomieu*), and spotted bass (*Micropterus punctulatus*). Brook trout model equations were used to estimate TBW and DM in all species of fish. The sum of these terms (TBW and DM) was used to approximate a predicted wet mass of each fish and were subsequently correlated with the actual wet mass of the fish. Actual water and dry masses were measured following Association of Official Analytical Chemists (1990) methods.

Physical response to BIA methods

To test subject responses to BIA procedures, 20 additional hatchery brook trout were placed individually into 37-L aquaria and fed a maintenance ration of fly larvae (Gruco Inc., Hamilton, Ohio). Half of the fish were randomly assigned to a treatment group and measured for impedance and the other assigned to a control group that were not measured for impedance. Regardless of group, once weekly for 3 weeks, each fish was anesthetized and measured for length and mass with the additional measurement of impedance taken from the treatment group fish. Following measures, fish were immediately returned to the aquaria. Observations of bleeding were noted immediately following BIA measures, and changes in color, feeding, swimming, and bruising were observed once daily. A flashlight was used to visually determine bruising and color, while feeding and swimming were scored as "not swimming, listless" or "not feeding". A dichotomous variable score (0 or 1; i.e., 0 = no bruising, 1 = bruising) was used to monitor basic observations. This repeated-measures experiment was analyzed with a logistic regression.

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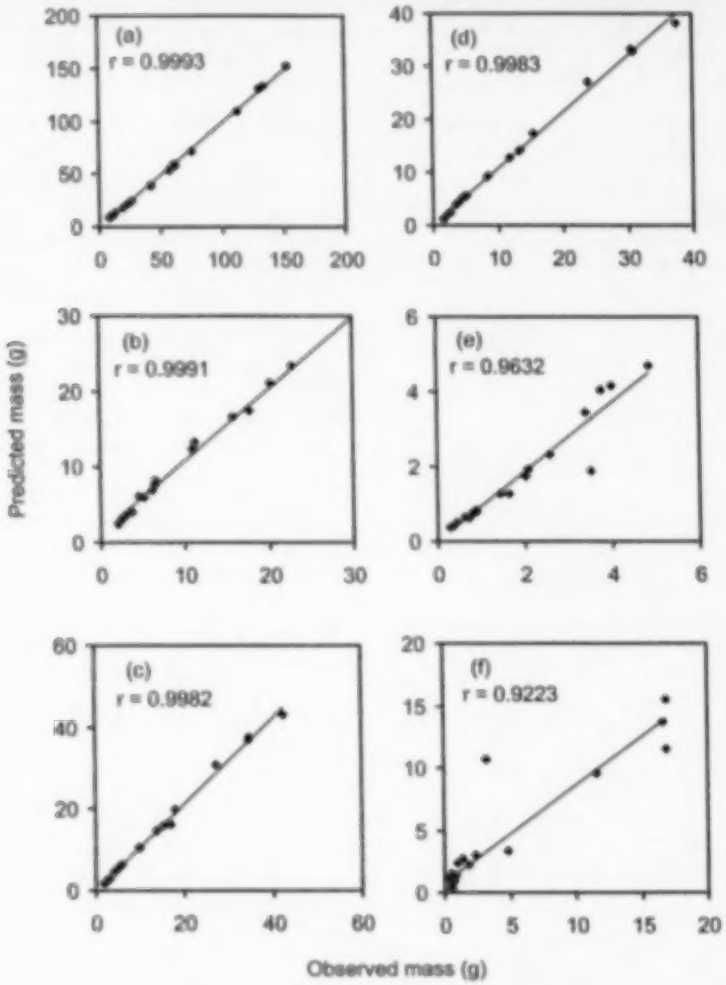
Fig. 2. Correlation analysis of predicted and actual values for the brook trout (*Salvelinus fontinalis*) validation group (n = 20) including (*a*) total body water, (*b*) dry mass, (*c*) fat-free mass, (*d*) total body protein, (*e*) total body ash, and (*f*) total body fat.



Results

Strong linear relationships were found between independent variables calculated from BIA values and laboratory-derived body composition values. Independent and dependent variables with associated correlation strengths (r values) are as follows: resistance (in series) with TBW (r = 0.9872), reactance (in parallel) with DM (r = 0.9862), combined resistance and reactance (in series) with FFM (r = 0.9873), resistance (in series) with TBP (r = 0.9863), resistance (in parallel) with TBA (r = 0.9864), and capacitance with TBF (r = 0.9779) (Table 2).

Tests of the linear regression models using the validation group of brook trout showed that the models were good predictors of all body composition values. Predicted and actual values for all body composition parameters were highly correlated ($p < 0.0001$) with r scores ranging from 0.9223 to 0.9993 in TBF and TBW, respectively (Fig. 2). The F value tests ($p < 0.0001$) and residuals reflected a linear relationship between impedance values and the predicted values (Fig. 2). Correlations between predicted and observed values in all proximate composition categories indicated a strong linear relationship with values not differing from 1:1 (Fig. 2).

Linear regression models developed for brook trout were also good predictors of DM, TBW, and mass across all species of fish tested from the Kanawha River. Predicted values of DM, TBW, and mass were also strongly correlated with actual values (Fig. 3; Table 3). DM predicted and actual values were highly correlated, with r ranging from 0.8584 in redbreast sunfish to 0.9921 in gizzard shad. Predicted and actual values of TBW were highly correlated, with $r >$ 0.9500. Predicted and actual total mass values were highly correlated, with $r > 0.9900$ for all species.

In repeated-measures laboratory experiments, brook trout showed little response to being measured with BIA. Measured parameters of swimming, feeding, bleeding, and color were not significantly different between the two groups (applied BIA and not). Bruising was a significant (logistic regression $p < 0.0001$) response to BIA but was slight and lasted only 2 days (Table 4).

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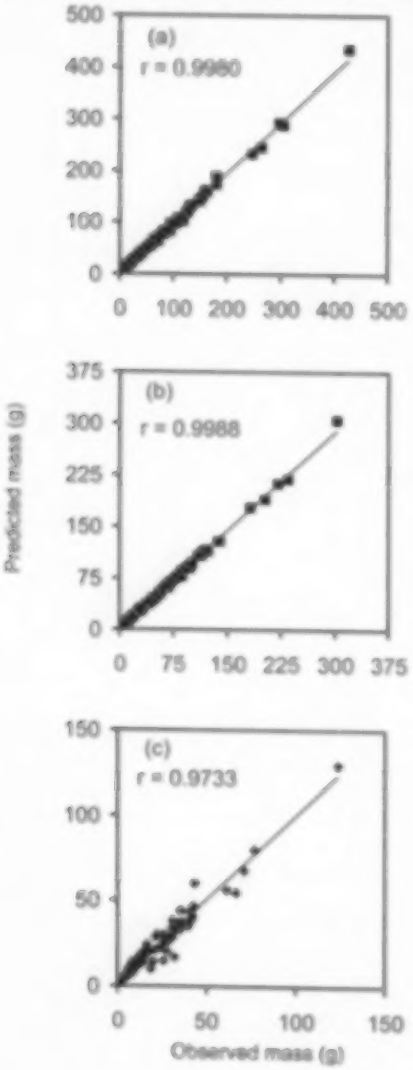
Table 4. Number of negative responses (e.g., loss of color, not feeding or swimming, bruising, bleeding) of individual brook trout (*Salvelinus fontinalis*) that underwent bioelectrical impedance analysis (BIA) compared with those that received no BIA (control).

Treatment	*n*	Color	Feeding	Swimming	Bruising	Bleeding
Control	210	4	22	3	1	0
BIA	210	0	13	1	110[a]	3

[a]Significant ($p < 0.0001$).

Fig. 3. Correlation analysis of predicted and actual values for the warmwater species validation group ($n = 81$). Predicted values of (*a*) wet mass, (*b*) dry mass, and (*c*) total body water were calculated using the existing brook trout (*Salvelinus fontinalis*) model.



Discussion

BIA models provided a means of estimating body composition in brook trout and a group of warmwater fish species with a high degree of predictability ($R^2 > 0.8507$). Additional experiments showed that the methods of BIA are nonlethal and appear to produce little measurable effect on fish health or behavior. The portability of the BIA unit makes this a useful method that can be utilized in the field. The BIA instrument is a small handheld unit that is powered by a 9-V battery. Once a model is built, BIA estimations are derived from three measurements: resistance (measure of extracellular resistance), reactance (measurement of "celled" mass), and distance between detecting electrodes. These three measurements are used to predict TBW, DM, FFM, TBP, TBA, and TBF and are obtained on live, anaesthetized organisms in about the time it takes to measure live mass.

The strong predictability and accuracy found in this fish data are a result of the body geometry of fish being more simplified than the higher vertebrates used in previous BIA studies (Berg and Marchello 1994; Marchello et al. 1999). More specifically, trout have a fusiform geometric shape that approximates a cylinder with a majority of the mass located in the thorax (Jobling 1995). The thorax accommodates all major body composition components and likewise is the main region for hypertrophic or hypotrophic growth. Since volume is proportional to the impedance and the length between detecting electrodes (see eqs. 1–4 in the Materials and methods section), a single impedance measurement represents the whole body and likewise compositional changes that occur within it. If the majority of mass is not located within a single volume (as with many higher vertebrates), it must be distributed into limbs or appendages. Since each limb or appendage has its own volume and tissue heterogeneity, a single measurement of impedance cannot represent the whole organism (e.g., two different-sized volumes with identical composition would have different impedance measurements) (Keller et al. 1993). Likewise, measuring impedances for each separate volume and combining them would result in complex methodology and conversely a complex model with error propagations occurring with each volume. For a single measurement of impedance to be representative of the whole body, simple body geometry with the majority of the mass located in one volume is desirable.

A validation of the brook trout BIA model with an independent BIA data set showed that the models accurately predicted actual values ($r > 0.9632$) except for lower predictability of fat mass ($r = 0.9223$). The reasoning for the weaker correlations for fat mass may be explained by electrical resistivity properties. The nature of current division described by Ohm's law and Kirchhoff's rules dictates that current will pass through an entire circuit, but the path with the least resistance will carry more current. Fat deposits (*i*) are concentrated in the ventral gut region of the fish and (*ii*) have a higher resistance than other visceral or somatic tissues. Since electrode placement was in the dorsal region, and resistance is higher in fat, it is possible that the current

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does not represent the fat that is located in the lower ventral region. All other parameters such as TBW and TBP are more systemic, have a lower electrical resistance, and therefore are better represented by impedance values in this study. This leads to the possibility of localized or tissue-specific BIA modeling via electrode placement.

Furthermore, strong linear relationships in the model predictions for a data set of warmwater species suggest that the brook trout model may predict compositional parameters for other species. This may be due to the similar geometric shapes found among the species of fish used in this study. The warmwater species validation group was consistent with geometric shapes of a cylindrical shape with the majority of the mass (body composition components) located within one volume, and predictions with the brook trout BIA model were strongly correlated with actual parameter estimations. Some of the variation between predictions with the brook trout model and observed values for the warmwater species, especially in the DM parameter, may have been due to regional tissue deposits. Determining error constitutes is outside the scope of this study, but the authors feel that lower vertebrates such as fish, amphibians, reptiles, and some mammals that fit the criteria mentioned above would be eligible for BIA modeling. The brook trout models used here were to test for model generality and we conclude that general relationships observed for brook trout measured with BIA will hold for other species regardless of size if geometry is similar.

The ability to precisely and nonlethally estimate proximate composition through BIA will permit increased precision in energy flow and proximate composition studies on spatial and temporal scales that were previously impractical. At the individual level, BIA will permit repeated measures on the same individual during the course of investigation, yielding better tracking of energetics components and improved precision in bioenergetic models. At the population level, BIA will permit assessment of condition of cohorts over time and permit detailed comparisons across cohorts and temporal and spatial scales, such as evaluating body conditional properties of highly migratory species at different points during migration. At the community level, BIA will permit the evaluation of growth and energy flow dynamics across species that may elucidate community dynamics that were previously unknown or permit correlation of body composition with outbreaks of disease. This approach also has potential for the nonlethal study of threatened or endangered species by utilizing models developed for closely related species. Finally, this could be applied to other taxa groups, particularly amphibians and reptiles, with similar results and uses as described here.

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Exploration of riffle-scale interactions between abiotic variables and microbial assemblages in the hyporheic zone

Lucie Sliva and D. Dudley Williams

Abstract: Molecular methods were applied to explore the composition of hyporheic bacterial assemblages and their possible interaction with interstitial physicochemical variables. Hyporheic flows on a riffle were manipulated (a downwelling zone was converted to an upwelling zone and vice versa) in order to examine the influence of vertical flow patterns on microbial assemblages. Bacterial assemblages were heterogeneously distributed throughout the riffle, the greatest similarity among them occurring at similar depths rather than in areas of similar flow regimes (upwelling versus downwelling). There was no correlation between the measured physicochemical variables and the microbial assemblages, with the exception of depth and sediment particle size. The flow manipulation in the original downwelling zone appeared to have had an effect on the microbial assemblages, although this influence may have been primarily due to a change in temperature in the manipulated region rather than an alteration of the vertical-flow direction per se.

Résumé : Des méthodes moléculaires nous ont servi à explorer la composition des peuplements hyporhéiques de bactéries et leurs interactions possibles avec les variables physicochimiques du milieu interstitiel. Nous avons manipulé l'écoulement hyporhéique dans une zone de rapides (une zone d'enfoncement des eaux a été transformée en zone de remontée et inversement) afin d'étudier l'influence des patterns d'écoulement vertical sur les peuplements microbiens. Les peuplements microbiens se répartissent de façon hétérogène dans la zone de rapides et les plus fortes similarités entre les peuplements s'observent aux profondeurs semblables plutôt que dans les zones à régime d'écoulement similaire (enfoncement ou remontée). Il n'y a pas de corrélation entre les peuplements microbiens et les variables physicochimiques mesurées, sauf la profondeur et la taille des particules du sédiment. La manipulation de l'écoulement dans la zone originale d'enfoncement de l'eau semble avoir eu un effet sur le peuplement microbien, bien que l'influence puisse peut-être s'expliquer plus par le changement de température dans la région manipulée que par le changement de l'écoulement vertical en lui-même.

[Traduit par la Rédaction]

Introduction

The hyporheic zone is subject to a unique and dynamic set of physicochemical conditions unlike those found in the more intensively studied surface- or ground-water habitats. Of particular interest are the two regions of contrasting hydraulic regimes within a single riffle, the downwelling and upwelling areas. The downwelling area generally occurs at the head of a riffle, where oxygen- and nutrient-rich surface water percolates downwards and is subject to larger daily and seasonal fluctuations in temperature, nutrient levels, and organic matter quality and quantity (Boulton et al. 1998). Downwelling of surface water in this area is caused by high local hydraulic pressure induced by current flow. The upwelling area is generally characterized by oxygen-poor groundwater moving through the alluvial sediment into the stream, as well as surface water that has travelled the length of the riffle to reenter the stream. This area occurs at the downstream end of the riffle, where increasing stream depth produces a low-pressure zone, and is characterized by more constant temperature and nutrient and organic-matter levels. The contrasting hydrological and chemical characteristics of the downwelling area tend to be more conducive to aerobic processes such as respiration, nitrification, and methane or sulphide oxidation, whereas those of the upwelling area are more conducive to anaerobic processes such as denitrification, ammonification, and sulphate reduction (Hendricks 1993).

Within the hyporheic zone, microbes are thought to account for the bulk of the living biomass, which in most hyporheic ecosystems is dominated by heterotrophic bacteria (Findlay and Sobczak 2000). Despite this, microbial patterns and processes within the hyporheic zone are poorly known compared with those in other aquatic habitats (Findlay and Sobczak 2000). In the few existing studies, researchers have observed higher bacterial abundances, activity, and bacterial respiration in downwelling zones and shallow sediments than in upwelling zones and deeper sediments (e.g., Findlay and Sobczak 1996; Hendricks 1996). Henry and Fischer (2003), for example, have found the downwelling zones of sandbar edges in an Arizona creek to contain communities

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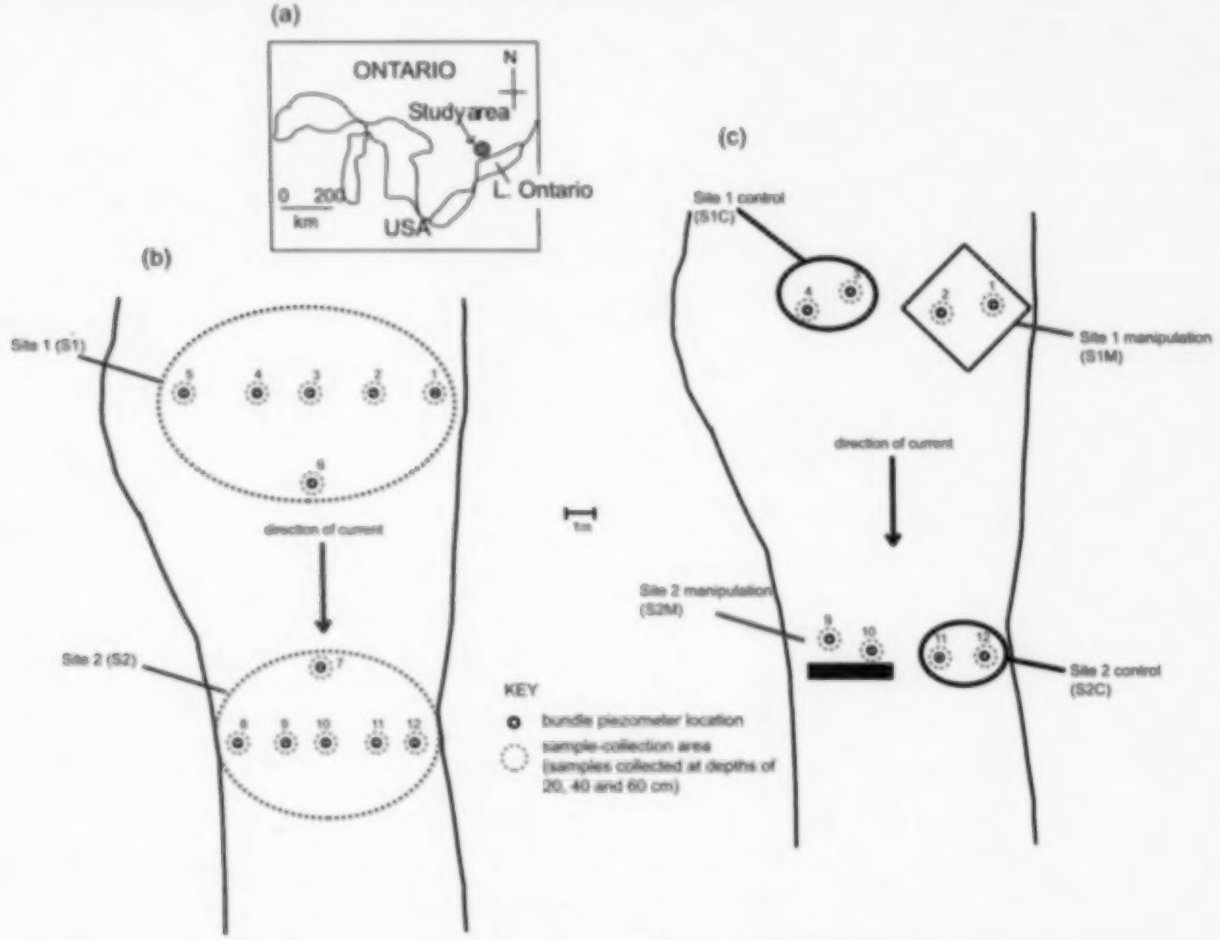
Fig. 1. (*a*) Location of the study area, northeast of Guelph, Ontario (43°43′N, 80°16′W). (*b*) Distribution of the pre-manipulation sample-collection sites. (*c*) Distribution of the manipulation and post-manipulation sample-collection sites (C, control treatment; M, manipulation treatment; S1, originally downwelling zone; S2, originally upwelling zone).



with abundant nitrogen-fixing cyanobacteria, while non-nitrogen-fixing taxa were predominant at the upwelling edges. However, other studies have reported no variations in these parameters between the two zones (Claret et al. 1998; Franken et al. 2001). Besides hydrology, physical characteristics of subsurface sediments, such as sediment surface area, have also been noted to influence bacteria (Leichtfried 1985).

Despite the importance of manipulative studies to testing and establishing the role of physicochemistry in determining the diversity and structure of hyporheic biological assemblages, very few field-scale manipulative studies exist (i.e., Richards and Bacon 1994; Boulton and Foster 1998). To our knowledge none has attempted to use field manipulations to look at the influence of physicochemistry in structuring the microbial assemblages in the hyporheic zone.

The objectives of this study were (*i*) to explore the composition of hyporheic bacterial assemblages; (*ii*) to examine associations between interstitial physicochemical variables and microbial assemblages; and (*iii*) to test the strength of influence of a suspected major variable (hyporheic flow) on microbial assemblages by experimentally reversing upwelling and downwelling areas in a riffle.

Methods

Study site

The study site was a pool–riffle–pool segment of the Speed River northeast of the city of Guelph, Ontario (43°43′N, 80°16′W), situated about 5 km from the spring source (Fig. 1*a*). The local geography is composed of undulating hills, glacial spillways, and swampy depressions with the river flowing through an average gradient of 2 m·km^{-1} (Chapman and Putnam 1966). The river is 4–6 m wide at the site and varies in depth between around 0.15 and 0.35 m. The Speed River is characterized by peak flow during the spring snowmelt and reduced flow during the rest of the year. The summer base-flow discharge is about 0.1 m^3·s^{-1}, and in the winter this discharge increases by a factor of 2–3. To a depth of about 30 cm, the substrate is primarily composed of gravel intermixed with silt, sand, and a few larger

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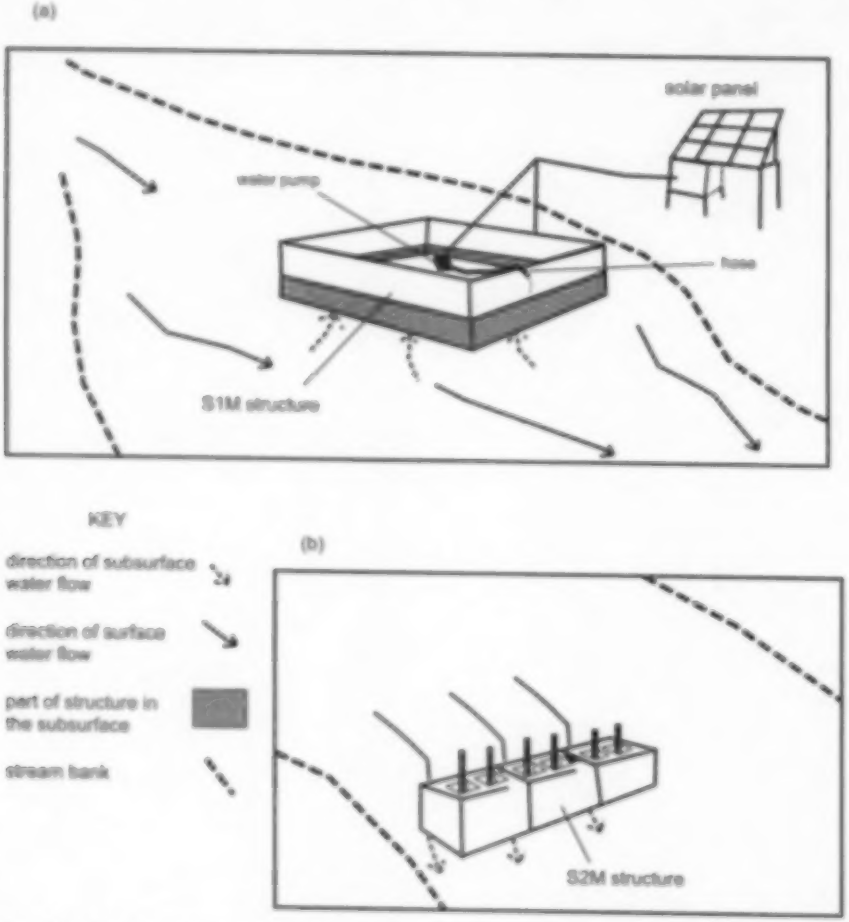
Fig. 2. Experimental flow-manipulation setup. (*a*) S1M is the upstream manipulation area, where a downwelling zone was converted to upwelling. (*b*) S2M is the downwelling manipulation area, where an upwelling zone was converted to downwelling. The scale is not accurate.



dolomite slabs. Below this, the substrate is of low heterogeneity and is dominated by medium and fine sands. The vegetation on the north bank of the river is composed of eastern white-cedar (*Thuja occidentalis* L.), whereas that on the south bank consists primarily of open fields with pockets of deciduous trees.

Flow manipulations

The flow-manipulation portion of this study involved the alteration of a portion of the riffle upwelling area into a downwelling area and vice versa. The upwelling and downwelling areas were about 11 m apart (Fig. 1*b*). The redirection of subsurface flows in the initially downwelling area (head of the riffle) consisted of isolating a 6.25-m^2 area with an open (top and bottom), galvanized-sheet-metal, waterproof structure (having 1-m high walls), similar in function to a caisson used to provide a working space for construction projects below ground or under water (Fig. 1*c*). This structure was buried 40 cm in the streambed. Water was pumped continuously out of the caisson with a submersible water pump powered by a photovoltaic system, at a rate that maintained a water surface within the structure that was lower than that of the outside stream (S1M structure; Figs. 1*c* and 2*a*). The extent of this flow reversal was measured by two sets of mini piezometers installed at three depths (20, 40, and 60 cm) within the structure. The distance between the replicate piezometer sites was 1.5 m. The redirection of subsurface flows in the initially upwelling area (downstream end of the riffle) involved constructing a cement-block barrier secured by metal poles that spanned half the width of the river (S2M structure; Figs. 1*c* and 2*b*). This was expected to increase the hyporheic hydraulic head upstream of the structure and lower the hydraulic head downstream of the structure, which caused surface water to infiltrate down into the sediments, thus producing a local downwelling zone similar to natural downwellings produced by logs positioned perpendicular to current (Baxter and Hauer 2000). Since the number of samples that could be collected was limited by the size of the area that was practicable to manipulate, the replication of samples was limited to two sets of samples per depth (each sample set corresponds to sediments collected at 20, 40, and 60 cm) within the ma-

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nipulated areas. Two sets of control samples were also collected at each of these depths within the upstream and downstream areas that were not manipulated (Fig. 1*c*). This resulted in 6 samples in each of the two manipulated and control areas and a total of 24 samples collected during the manipulation period. The distance between the upstream manipulation (S1M) structure and downstream control (S2C) area was 8 m. The distance between the S1M area and upstream control (S1C) area was 2 m. The distance between the downstream control and manipulation areas was the same. The manipulation continued for 1 month, as the recovery of the invertebrate community was also studied (unpublished), and it takes about 1 month for hyporheic invertebrate communities at this site to recover after disturbance (Williams and Hynes 1976).

Sample collection and physicochemical analyses

The area was sampled three times during the summer and autumn of 2001. The pre-flow-manipulation samples were collected during the second week of July. The flow-manipulation samples were collected during the first week of October after the hyporheic flows had been reversed for 1 month. The post-flow-manipulation samples were collected during the first week of November, 1 month after the manipulation structures had been removed and the system returned to pre-manipulation status.

Hyporheic sediment samples were collected 20, 40, and 60 cm below the surface of the riverbed with a standpipe corer (n = 12 at each depth for the pre-manipulation set and n = 10 at each depth for the manipulation and post-manipulation sets; see Fig. 1 for sampling locations). This corer collects 25 cm^3 of sediment per sample (Williams and Hynes 1974). The samples were collected within 30 cm of corresponding piezometers that were used to measure hydraulic heads and to collect water samples for chemical analysis. The sediment samples used for microbial analysis were washed out of the standpipe corer chamber with sterile, filtered water into sterile plastic bags, homogenized by thorough mixing, and transported to the laboratory on ice. Immediately upon return to the laboratory, 10 g of the sediment was removed from each sample for microbial analysis and stored at –80 °C.

The hydraulic heads were measured on site with an electronic water-level indicator. The vertical hydraulic gradient (VHG) was determined by direct determination of the piezometric head relative to the stream surface, which provided an estimate of the potential strength of the hydrological exchange occurring within the upwelling and downwelling zones (Boulton et al. 1998). VHG was determined by the following equation: VHG = dh/dl, where dh is the difference in head between the water surface in the piezometer and the level of the stream surface (hydraulic-head differential, in centimetres), and dl is the depth from the substrate surface to the first opening in the piezometer's side wall (Baxter and Hauer 2000). VHG is a unitless measure that is positive under upwelling conditions and negative under downwelling conditions.

Water samples at depths of 20, 40, and 60 cm were withdrawn from each piezometer with a peristaltic pump and Tygon™ R3603 tubing. Water standing in the piezometers was discarded, after which a 250-mL sample was taken for on-site analysis of temperature, dissolved oxygen (DO), redox, conductivity, and pH, using a Hydrolab Scout H_20 multiprobe (Hach Co., Loveland, Colorado). Another set of 250-mL samples was taken from the piezometers, stored on ice, and transported to the laboratory, where they were immediately filtered (Gelman A/E/ 1.0-µm glass-fiber filters) and analysed for NO_3-N and NH_4-N, using a Hach™ DR2000 spectrophotometer (Hach Co.), within 24 h of collection. Water samples to be used for dissolved organic carbon (DOC) analysis were filtered with Gelman A/E/ 0.45-µm glass-fiber filters, acidified to pH 3.0 with sulphuric acid, and analysed at Environmental Engineering, Department of Civil Engineering, University of Toronto, Toronto, Ontario. Glassware and sample bottles were acid-washed in 1:6 HNO_3 and rinsed six times in distilled water and five times in Nanopure™ deionized water. Particulate organic matter (POM) in each sediment sample was quantified by the loss of weight on ignition method (Nelson and Sommers 1996) after removing hyporheic fauna. The sediment samples were first oven-dried at 105 °C for 24 h. Dried samples were weighed, ashed at 400 °C for 14 h, and weighed again. The amount of organic matter in the core sample per unit weight of sediment was determined by dividing the loss of weight on ignition by the weight of the core sample.

Microbial analysis

Two methods were used to extract microbial DNA directly from the hyporheic sediments. The first was with the Ultra Clean Soil Kit (Mo Bio Laboratories Inc., Carlsbad, California), which was used to extract DNA from the pre-manipulation and manipulation samples. It was later determined that although the DNA extracted with this method was very clean, not requiring any further purification steps, it provided a poor yield compared with a subsequently modified (Zhou et al. 1996) method, which was applied to the postmanipulation set of samples.

Extracted DNA was amplified using universal bacterial primers 63F (5′-CAGGCCTAACACATGCAAGTC-3′) and 1389R (5′-ACGGGCGGTGTGTACAAG-3′) (Osborn et al. 2000), which were labelled at the 5′ end with the phosphoramidite dyes 6-FAM and HEX (Sigma–Genosys, Oakville, Ontario), respectively. All reagents were prepared as a master mix before the addition of the template DNA. One nanogram of template DNA was amplified in a 50-µL reaction consisting of 5 µL of 10× polymerase chain reaction (PCR) buffer, 2 U of Biotools DNA polymerase (1 U·µL^{-1}, BandM Labs, Madrid, Spain), 0.8 µmol·L^{-1} each primer, 0.2 mmol·L^{-1} dNTP mix (dATP, dCTP, dGTP, dTTP; Roche Diagnostics Corp., Indianapolis, Indiana), 3 mmol·L^{-1} MgCl, and 5 µL of MasterAmp™ 10× PCR enhancer. The cycling conditions consisted of initial denaturing at 94 °C for 2 min, followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min and 72 °C for 2 min. This was followed by a final extension step of 72 °C for 10 min.

Terminal restriction fragment length polymorphism (T-RFLP) analysis was used to assess the degree of microbial-assemblage similarity, and is based on digestion by restriction endonuclease of fluorescently end-labelled PCR products. The restriction endonuclease used in this study

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was *Msp*I (New England Biolabs Inc., Beverly, Massachusetts). For the *Msp*I restriction digestion reactions, 5 µL of buffer, 1 µL (20 U) of restriction enzyme, 20 µL of PCR product, and 24 µL of sterile distilled H_2O were combined. The mixture was incubated at 37 °C for 4 h. Ten microlitres of the digested DNA was run on 3% agarose gel stained with ethidium bromide to check if the products were fully digested. Digestion was deemed complete because (*i*) 1500-base-pair (bp) fragments were never seen; (*ii*) incomplete digestion is normally encountered only when there is excess DNA in the reaction, which was never the case with our samples; and (*iii*) good result replication suggests that digestion was complete. Further, the primers have been tested in many other studies, and annealing to any gene section other than 16S (e.g., 18S) is not observed when the correct PCR protocol is applied. The remainder of the digested DNA was sent to the Molecular Supercentre, University of Guelph, Ontario, where the digested products were mixed with a DNA size standard (size range between 30 and 600 bp), itself labelled with a distinct dye, and the DNA fragments were analysed on an ABI 377 automatic sequencer (Applied Biosystems Inc., Fredmont, California).

A clustering algorithm was used to align the terminal restriction fragment profiles, which corresponded to specific microbial taxa or operational taxonomic units (OTU). This was done using GeneScan® software (version 2.1; Applied Biosystems Inc. 2000) and by setting the error that determines the fragment size to some predetermined value. Bacterial taxa will be referred to as OTUs throughout this paper, since each fragment does not necessarily correspond to a specific phylogenetic level, although it does roughly correlate with the level of genus. In this study, the error was set to ±1.0 bp, and thus OTUs that differed by less than 1.0 bp were considered identical and clustered together. All fragments within such a defined cluster were then assigned the average value of the sizes within the cluster. The minimum peak detection threshold value was set to 100 fluorescence units. Peaks not present in the pre-manipulation samples were removed from the flow-manipulation sample set, so that portions of the two assemblages compared were kept the same.

T-RFLP is based solely on fragment size, and direct comparison with a sequence database such as the Ribosomal Database Project II (RDP II, version 8.0, University of Michigan) is possible (Marsh et al. 2000). The T-RFLP analysis program of the database was used to perform an in silico digestion (all bacterial sequences in the database were used in a model digestion) with *Msp*I of all the RDP sequences amplifiable by the 63F primer. The results of this analysis were compared with the terminal-fragment sizes obtained from *Msp*I digestion of the hyporheic assemblage DNA.

Data analysis

Summary statistics, correlations, analysis of variance (ANOVA), and nonparametric analyses were performed with StatView® for Windows, version 5.0 (SAS Institute Inc. 1999). Microbial data were log $(x + 1)$ transformed where this improved normality. Physicochemical variables were log $(x + 1)$ transformed to improve normality and standardized to zero mean and unit variance (Jongman et al. 1995). When differences in physicochemical and biological variables among depths were analysed, samples belonging to the same zone (upwelling versus downwelling) were grouped to increase the number of replicates at each depth. Similarly, samples from similar depths were grouped when differences between zones were analysed to increase the number of replicates in each zone. Principal components analysis was used to preselect physicochemical variables of importance for further analysis in order to decrease the number of variables to be used and to reduce intercorrelations. Where multiple tests were performed (e.g., testing for differences among physicochemical variable for different depths and zones), Bonferroni correction was applied in order to avoid spurious positives. Multivariate statistical analyses were performed using Canoco® for Windows, version 4.0 (Centre for Biometry 1997). Detrended correspondence analysis was used to determine which multivariate, linear (regularized discriminant analysis), or unimodal (canonical correspondence analysis (CCA)) method to apply to the data sets (Jongman et al. 1995). Since all biological data displayed unimodal distributions (first gradient of detrended correspondence analysis >3), CCA was used to analyse patterns in relationships between the physicochemical and biological parameters. CCA looks for coefficients of environmental variables to obtain a site score that maximizes the variance of average position of species. It is a direct gradient analysis technique that constrains the extracted pattern of microbial assemblages to a linear combination of the measured environmental variables, and it is a proven, robust method for describing species–environment relationships (for a complete review see Palmer 1993). Unconstrained correspondence analysis (CA) was applied to the species data first to determine significant patterns in the assemblage before the analysis was constrained by environmental data (CCA).

Results

Physicochemical patterns

The decrease in hyporheic exchange from pre-manipulation to post-manipulation sampling periods could be observed as an overall increase in vertical hydraulic gradient and a decrease in DO, which signify an increased upward flow of groundwater towards the stream and a decreased contribution of stream water to hyporheic flows (Table 1). Notably, there was a large decrease in water temperature over the three sampling periods, which can be attributed to natural seasonal differences.

Before the manipulation, significant differences in temperature, ammonium, VHG, and conductivity between the S1 (downwelling) and S2 (upwelling) areas were seen (Table 1), values being lower in S1 than in S2. The observed pattern of temperature difference between S1 and S2 was opposite to what was expected, since at that time of the year, the groundwater was colder than surface water. This could be explained by diurnal stream temperature change occurring more rapidly than on-site chemistry analysis for that sampling period; S1 samples were analysed first, making them appear, on average, cooler than the shallow hyporheic samples sampled in S2. Smaller sample size as well as later sampling time during the day avoided this problem during subsequent sampling periods. Significant differences in

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Table 1. Comparison of average values (+1 standard deviation, in parentheses) of physicochemical variables among zones for each sampling period as determined by analysis of variance.

	VHG	DO ($mg \cdot L^{-1}$)	Temperature (°C)	pH	Conductivity ($S \cdot cm^{-1}$)	NH_4 ($mg \cdot L^{-1}$)
Before manipulation						
S1	0.004 (0.017)	5.09 (2.4)	17.3 (0.1)	7.5 (0.4)	0.493 (0.017)	0.008 (0.006)
S2	0.021 (0.015)	2.74 (1.04)	18.3 (0.5)	7.3 (0.1)	0.529 (0.036)	0.037 (0.035)
n	16	18	18	18	18	18
F	8.291		12.03		7.368	9.107
p	0.007	ns	0.01	ns	0.010	0.005
S1M	0.122 (0.07)	2.06 (0.54)	14.8 (0.9)	7.4 (0.08)	0.527 (0.016)	0.172 (0.030)
Manipulation						
S1C	0.003 (0.015)	3.84 (2.66)	13.1 (0.4)	7.7 (0.21)	0.521 (0.006)	0.142 (0.115)
S2M	0.024 (0.028)	2.37 (1.62)	11.6 (0.5)	7.5 (0.09)	0.532 (0.007)	0.187 (0.050)
S2C	0.02 (0.005)	2.37 (0.22)	12.3 (0.4)	7.4 (0.02)	0.515 (0.003)	0.14 (0.100)
n	12	12	12	12	12	12
F	13.23		34.39			
p	<0.001	ns	<0.001		ns	ns
After manipulation						
S1	0.050 (0.02)	2.9 (0.9)	7.0 (0.6)	7.2 (0.1)	0.498 (0.039)	0.160 (0.07)
S2	0.080 (0.06)	2.8 (0.74)	6.6 (0.8)	7.4 (0.1)	0.491 (0.009)	0.160 (0.06)
n	18	18	18	18	18	18
F				12.14		
p	ns	ns	ns	0.002	ns	ns

Note: Depths (20, 40, and 60 cm) were combined to increase the sample size. S1M, upstream manipulation area (with control area S1C); S2M, downstream manipulation area (with control area S2C); VHG, vertical hydraulic gradient; DO, dissolved oxygen; ns, not significant.

Table 2. Comparison of average values (+1 standard deviation, in parentheses) of physicochemical variables (three particle sizes, DO, and temperature) at three depths below the river bed for each sampling period.

	Sediment particle size				
Depth (cm)	>2 mm (%)	<600 µm (%)	<250 µm (%)	DO ($mg \cdot L^{-1}$)	Temperature (°C)
Before manipulation					
20	26 (6)	41 (12)	16 (8)	4.63 (2.42)	18.1 (1.0)
40	24 (6)	47 (9)	21 (5)	3.51 (2.06)	17.8 (0.8)
60	19 (9)	56 (12)	56 (13)	3.35 (1.99)	17.6 (0.9)
n	12	12	12	12	12
F		8.266			
p	ns	0.001	ns	ns	ns
Manipulation					
20	27 (6)	36 (8)	12 (3)	4.03 (2.40)	12.8 (1.7)
40	29 (5)	39 (5)	17 (4)	2.16 (0.50)	13 (1.2)
60	24 (8)	42 (10)	18 (6)	1.97 (0.52)	13 (0.8)
n	6	6	6	6	6
F			4.866		
p	ns	ns	0.01	ns	ns
After manipulation					
20	27 (4)	35 (6)	14 (3)	3.85 (1.32)	6.2 (0.4)
40	28 (6)	39 (6)	18 (4)	2.88 (0.67)	6.8 (0.5)
60	29 (4)	40 (5)	19 (3)	2.48 (0.30)	7.4 (0.7)
n	12	12	12	12	12
F		7.333	4.694	45.6	5.82
p	ns	0.003	0.01	<0.001	0.008

Note: Only values that differed significantly among depths, as determined by analysis of variance, are shown. Sites were combined to increase sample size. DO, dissolved oxygen; ns, not significant.

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means among depths occurred for the sediment size fraction <600 µm, which decreased with depth (Table 2).

During the flow-manipulation period, significant differences in VHG, pH, and temperature were found between the control areas of the two sites (Table 1). Significant differences in VHG, temperature, and pH were also found between the S1 control and manipulation areas. The S1 control area (S1C) was generally downwelling, with lower VHG and temperature and higher pH. In S2, the control area (S2C) had a significantly lower pH and higher temperature and conductivity than the manipulated area (S2M). The only significant differences among depths during this sampling period were observed for DO, which decreased with depth, and sediment size fraction <250 µm, which increased with depth (Table 2).

During the post-manipulation sampling period, the only significant physicochemical difference between the sites was in temperature, which was higher in S1 (Table 1). The VHG values showed strong upwelling at both sites, and the difference in VHG between the upstream and downstream sites was not significant. Significant differences in physicochemical values among depths were observed for temperature, DO, and sediment size fractions <250 and <600 µm (Table 2). Temperature increased with depth, which corresponded to cooler surface water than groundwater at this time of year. The proportion of the small sediment fractions increased with depth below the streambed.

Microorganisms

The total numbers of OTUs used in further analyses of the pre-manipulation, manipulation, and post-manipulation data sets were 73, 70, and 95, respectively. Fewer than 50% of the OTUs in all sampling periods had corresponding fragment sizes derived from the model restriction digests of RDP II sequenced bacteria. Most of the hyporheic OTUs also present in the model digestion were associated with aerobic phylotypes. Many of the OTUs were associated with more than one phylotype. All phylotypes were gram-negative and belonged to the following three groups: Cytophaga, alpha-Proteobacteria, and gamma-Proteobacteria. Although taxa belonging to the subgroup Cytophaga appeared in both the pre-manipulation and manipulation sample sets, none was present in the post-manipulation samples. The OTUs that had the largest abundance values, as measured by fluorescence intensity, and their associated RDP phylotypes are shown in Table 3.

The results of an unweighted pair group method using arithmetic averages (UPGMA) cluster analysis are shown in Fig. 3. The pre-manipulation samples did not include five of the 60 cm depth samples and three of the 40 cm depth samples, since not enough amplifiable DNA could be extracted from them with the technique used at the time. Moreover, a low quantity of extractable DNA from deeper sediments was common to all of the samples analysed. The assemblages varied from being less than 20% to more than 90% similar, as measured by Pearson's correlation coefficient. There was an overall tendency for some samples from similar depths to cluster together for all three sampling periods. There was no clustering of samples originating from the same site or treatment during the manipulation.

Microbial richness, as measured by total number of OTUs per site, was compared among sites, depths, and treatments. No significant differences occurred except after the manipulation, when richness was greater in S2 than in S1 (Mann–Whitney test, $n = 12$, $p = 0.01$). No correlation was found between bacterial OTU richness and physicochemical variables before and after the manipulation. During the manipulation, OTU richness was positively correlated with temperature and VHG (Spearman's rank correlation, $r = 0.519$ and 0.573, $p = 0.0127$ and 0.006, respectively).

Although CCA of the log-transformed microbial data and the standardized physicochemical data showed that all CCA axes were able to account for a relatively large proportion of variation in the microbial data (45%–58%; Table 4), none of this was significant as tested by a Monte Carlo permutation test. Analysis of the pre-manipulation microbial data showed that the first species axis was a gradient of sediment size fractions <250 µm and >2 mm and POM ($r = 0.82$, –0.47, and –0.41, respectively), and the second axis a gradient of temperature and DO ($r = 0.31$ and 0.56, respectively; Fig. 4*a*). No clear separation of sites or depths was observed along either of the first two axes. There were several OTUs associated with increasing DOC (e.g., 395, 135, 274, and 449 bp); however, only one could be identified (104 bp belonging to gamma-Proteobacteria) on the RDP database. All the CCA axes could explain 45% of the variation in microbial-assemblage data (Table 4). Depth was not as well represented in this analysis because very few 60 cm depth samples could be included.

For the flow-manipulation data, CCA analysis showed that the first species axis, gradient of depth, pH, and DO ($r = -0.64$, 0.53, and 0.49, respectively), was the most important in determining variability in microbial assemblages (Fig. 4*b*). The second axis was a slight gradient of VHG and temperature ($r = -0.36$ and –0.34, respectively). There was a slight separation of deeper and shallower samples along the first axis, with shallower samples associating with increased gradient of DO, pH, and DOC. Most OTUs were also associated with the increased values of these environmental variables. Fewer OTUs were associated with the second axis. CCA of this sample set could explain about 54% of the species gradient as determined by CA.

The post-manipulation microbial data were influenced by a gradient of sediment size fraction <250 µm, temperature, and pH along the first axis ($r = 0.61$, 0.46, and –0.52, respectively; Fig. 4*c*). The second axis was primarily a gradient of depth ($r = -0.76$). Once again, there was a slight separation of samples from different depths, this time along the second axis. Most of the OTUs were also spread out along this gradient of depth. CCA of the post-manipulation microbial assemblages could explain about 58% of the species gradient as determined by CA.

Discussion

The Speed River physicochemical environment

The dominance of hyporheic exchange across the riffle scale was gradually reduced over the sampling periods, being most distinct before the manipulation and disappearing completely after the manipulation. This agrees with previous studies of this section of the Speed River, which have shown

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Table 3. Most-abundant operational taxonomic units and their corresponding ribosomal database (RDP) phylotypes before the manipulation, during the manipulation, and after the manipulation.

Fragment length (bp)	RDP phylotypes	RDP group
Before manipulation		
402	*Sulphitobacter, Rhodobacter, Paracoccus, Roseobacter*	Alpha- and beta-Proteobacteria
404	*Paracoccus, Rhodovulum, Hyphomicrobium, Roseobacter, Rhodospirillum, Magnetospirillum, Sulfitobacter*	Alpha-Proteobacteria
405	*Hyphomicrobium, Paracoccus*	Alpha-Proteobacteria
406	*Rhodospirillum, Rhodovibrio*	Alpha-Proteobacteria
453	*Pseudomonas, Pseudoalteromonas, Alteromonas*	Gamma-Proteobacteria
454	*Pseudomonas, Pseudoalteromonas, Endobugula, Cytophaga*	Gamma-Proteobacteria, Cytophaga
457	*Pseudomonas, Pantoea, Cytophaga*	Gamma-Proteobacteria, Cytophaga
475	*Thiomicrospira*	Gamma-Proteobacteria
476	*Vibrio*	Gamma-Proteobacteria
During manipulation		
111	na	
173	na	
406	*Rhodospirillum, Rhodovibrio*	Alpha-Proteobacteria
454	*Pseudomonas, Pseudoalteromonas, Endobugula, Cytophaga*	Gamma-Proteobacteria, Cytophaga
455	*Pseudomonas, Pseudoalteromonas, Alteromonas, Flavobacterium*	Cytophaga, gamma-Proteobacteria
456	*Pseudomonas, Pseudoalteromonas, Pantoea*	Gamma-Proteobacteria
After manipulation		
113	Unnamed strain	Alpha-Proteobacteria
114	*Rhodocista, Methylosinus, Asticaccaulis, Pseudomonas*	Alpha-Proteobacteria
115	*Caulobacter, Methylocystis, Methylosinus, Pedomicrobium, Brevundimonas, Azospirillum*	Alpha-Proteobacteria
116	*Caulobacter, Azospirillum, Rhodothalasium*	Alpha-Proteobacteria
118	*Rhodovibrio*	Alpha-Proteobacteria
126	*Rhodobium, Rhodopseudomonas*	Alpha-Proteobacteria
127	A couple of unnamed clones	Alpha and beta Proteobacteria
271	*Psychrobacter*	Gamma-Proteobacteria
414	A couple of unnamed strains	Alpha-Proteobacteria
417	*Agrobacterium*	Alpha-Proteobacteria
419	*Stenotrophomonas*	Gamma-Proteobacteria
425	*Pseudomonas*	Gamma-Proteobacteria
468	*Vibrio*	Gamma-Proteobacteria
470	*Vibrio, Listonella, Photobacterium*	Gamma-Proteobacteria
471	*Vibrio, Photobacterium, Shewanella, Listonella*	Gamma-Proteobacteria
487	*Salinivibrio*	Gamma-Proteobacteria

Note: na, not available; bp, base pairs.

that true hyporheic exchange, and the associated chemical differentiation between upwelling (S2) and downwelling (S1) areas, occur mainly during the summer, when the flows are lowest (Storey et al. 2003).

The pre-manipulation physicochemical results show that S1 (the downwelling zone) had characteristics more typical of the stream surface water, characterized by lower conductivity and ammonium, which infiltrated the subsurface at the head of the riffle. Speed River surface water is characterized by higher nitrogen and DOC and lower ammonium values than interstitial water (R. Storey, Department of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Scarborough, ON M1C 1A4, unpublished data).

During the manipulation, there still was a differentiation in the direction of vertical flow and chemistry between the control areas, S1C having higher pH and temperature and lower VHG than S2C. Higher pH values in the downwelling area provide evidence for the presence of recently infiltrated surface water, which tends to be more alkaline than that in the hyporheic zone. As this water moves through the subsurface, pH decreases because it is linked to increasing CO_2 content produced by respiration along the flow line (Williams 1984). There was evidence of physicochemical differences between the control and manipulated areas. S1M had higher VHG and temperature and lower pH than S1C. This supports the success of flow reversal from downwelling to upwelling in S1M. The increase in temperature in S1M was an unintentional, and difficult to avoid, side effect of the manipulation structure itself. It was observed during the manipulation period that the slower moving water in the structure tended to be 3–4 °C higher than the river surface water.

There was also some evidence for altered flows in S2 (originally an upwelling zone). Although there was no significant difference between the VHG values of the control

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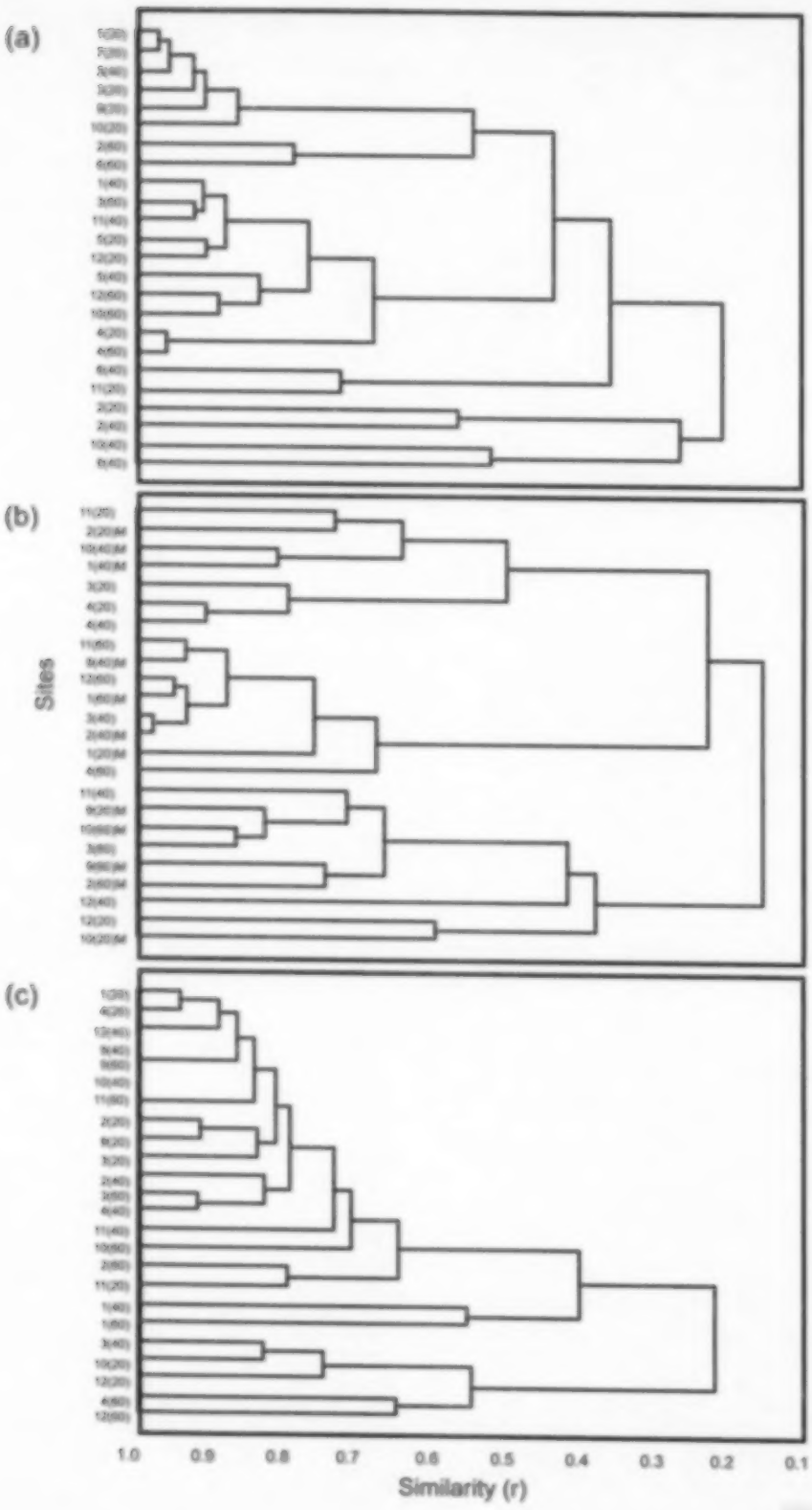

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Fig. 3. Unweighted pair group method using arithmetic averages (UPGMA) dendrograms based on comparisons of 16S rDNA (*Msp*I digested) terminal restriction fragment (TRF) patterns during the pre-manipulation (*a*), manipulation (*b*), and post-manipulation (*c*) sampling periods. The *y* axes correspond to samples taken from various depths on the riffle. Numbers in parentheses denote sampling depth. The *x* axes represent Pearson's correlation coefficient (*r*).

Table 4. Eigenvalues and microbial–physicochemical correlation coefficients for correspondence analysis (CA) and canonical correspondence analysis (CCA).

	Axis 1	Axis 2	Axis 3	Axis 4	Canonical or unconstrained eigenvalue
Before manipulation					
CA eigenvalue	0.311	0.269	0.212	0.187	
CCA eigenvalue	0.225	0.171	0.120	0.088	0.451
Taxon–environment correlation	0.905	0.868	0.871	0.912	
Manipulation					
CA eigenvalue	0.509	0.459	0.182	0.130	
CCA eigenvalue	0.320	0.238	0.116	0.095	0.535
Taxon–environment correlation	0.837	0.741	0.950	0.791	
After manipulation					
CA eigenvalue	0.249	0.098	0.067	0.042	
CCA eigenvalue	0.185	0.146	0.094	0.075	0.580
Taxon–environment correlation	0.901	0.938	0.951	0.874	

and manipulated areas, S2M had a higher pH than S2C, which is indicative of a greater influence on surface water in the manipulation area than in the control area. Although the temperature in the manipulation area was also significantly lower than in the control area of this site, the difference between groundwater and surface water at this time of the year was not large, thus this variable was not a good indicator of altered hyporheic flows.

Microbial assemblages

The number of different bacterial OTUs generated in this study is likely an underestimation of richness in the hyporheic zone, since the resolution of T-RFLP analysis is often reduced from the species level to that of higher order groups. Further, populations that are not numerically dominant but have a significant functional role are also not represented (Liu et al. 1997). This is because less dominant populations could fall below detection threshold levels of PCR as well as T-RFLP. Less than 50% of OTUs generated in this study were present in the RDP database after a model digestion was performed with *Msp*I. One problem with attempting to identify bacteria from natural environments with this method is that a significant proportion of sequenced bacteria in the RDP is skewed towards medically important taxa (Marsh et al. 2000). In addition, 16S rRNA genes from phylogenetically related organisms could result in the production of terminal fragments of identical size (Liu et al. 1997). For these reasons, one cannot positively identify phylogenetic groups or species based on the length of terminal fragments alone. The portion of the hyporheic assemblage that could be associated with organisms identified in the RDP database is gram-negative, mainly aerobic, and can be divided into three general groups, Cytophaga, alpha-Proteobacteria, and gamma-Proteobacteria. Since organisms belonging to these groups are highly diverse (Woese 1987), no further conclusion can be drawn about the functional role they might have within the hyporheic environment. Feris et al. (2003) have also used molecular methods (denaturing gradient gel electrophoresis (DGGE), cloning and sequencing, quantitative real time reverse transcriptase PCR (qPCR)) to study microbial assemblages in the hyporheic zone. As in our study, the majority of microbes they identified were gram-negative heterotrophic bacteria. In addition to alpha- and gamma-Proteobacteria groups, they observed beta-Proteobacteria, Cyanobacteria, and Nitrospina groups. In contrast to our study, they did not observe any microbes that belonged to the Cytophaga group. The lack of OTUs that had RDP phylotype equivalents belonging to the Cytophaga group in the postmanipulation sample set could have been caused either by seasonal differences or by differences between the molecular technique applied to the first two sample sets and that applied to the last sample set. The former is supported by Feris et al. (2003), who observed significant seasonal differences in hyporheic assemblages in a hyporheic zone. They also detected obvious differences among the assemblages in three different streams. Based on the information derived from our data, the next logical step in dissecting the composition of bacterial assemblages and determining its functional characteristics would be to use group-specific primers (e.g., see Richardson et al. 2002). In addition, several different restriction enzymes are usually needed to resolve species that are phylogenetically similar (Marsh et al. 2000). Studies using primers that target functional genes, such as *nif*H, which is carried by nitrogen-fixing organisms (e.g., Poly et al. 2001), would prove very useful in unravelling the functional roles of hyporheic assemblages.

Cluster analysis performed on the microbial data showed that assemblage composition within the hyporheic sediments

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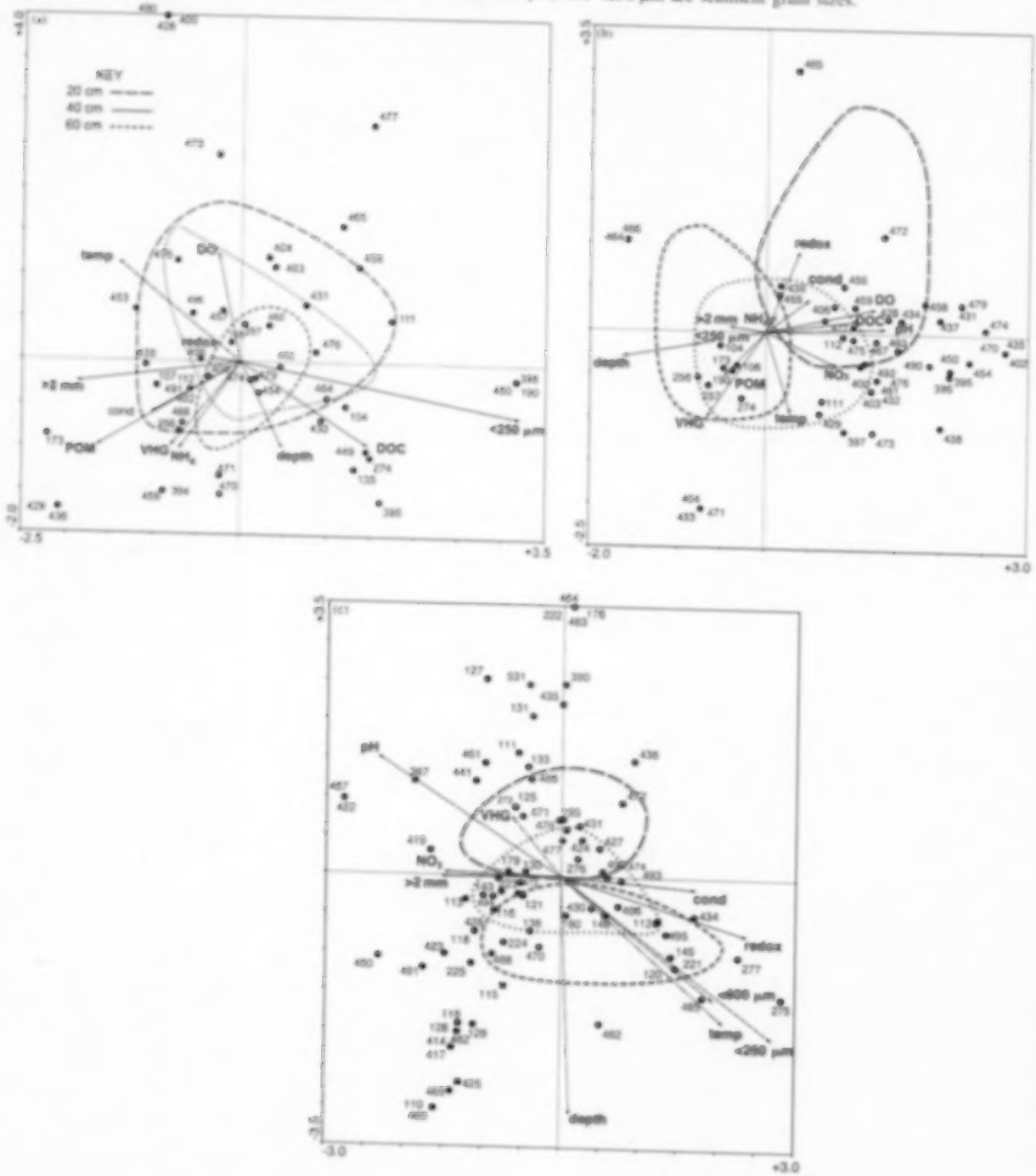
Fig. 4. Canonical correspondence analysis ordination plots of the pre-manipulation (*a*), manipulation (*b*), and post-manipulation (*c*) microbial assemblages and physicochemical variables. Numbers represent TRF sizes. The broken lines enclose samples from the same depth (not shown for clarity). DO, dissolved oxygen; DOC, dissolved organic carbon; temp, interstitial water temperature; VHG, vertical hydraulic gradient; POM, particulate organic matter; redox, redox potential; depth, depth in the sediment (20, 40, and 60 cm); NH_4, ammonium concentration; NO_3, nitrate concentration; >2 mm, <600 µm, and <250 µm are sediment grain sizes.



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of a single riffle was very heterogeneous and was not structured by the direction of vertical flows in the study riffle. This was tested by comparing areas of naturally contrasting VHG, as well as areas of manipulated vertical flows. This heterogeneity persisted throughout the three sampling periods as well as throughout the flow manipulation. Since the hyporheic environment in this study site is extremely patchy, with some subareas within the downwelling zone exhibiting characteristics of upwelling and vice versa (Storey et al. 1999), the lack of strong grouping among samples derived from similar sites within the riffle is not surprising. These patchy characteristics can be caused by local variations in flow caused by subsurface features such as large buried rocks and clay lenses (Brunke 1999). Feris et al. (2003) have also found microbial assemblages to be highly heterogeneous within a particular stream, although they only sampled upstream riffle locations.

It is possible that microbial assemblages are structured by environmental variation on a smaller scale than was measured here. Soil microbial populations, for example, display nonrandom heterogeneous distribution in soil matrices that result from differences between microenvironments in factors that have been shown to directly affect the survival of bacteria, such as water and nutrient availability (Ranjard and Richaume 2001). Furthermore, most bacteria in sediment habitats are attached to sediment particles to form highly complex biofilm assemblages (Costerton et al. 1995), which allow the microscale coexistence of a variety of bacterial types with contrasting metabolic requirements (Storey et al. 1999). Part of this complexity originates from steep diffusion gradients of nutrients and metabolites, thus spatial heterogeneity may result from differential growth of individual cells or microcolonies under a variety of local environmental conditions (Fenchel 2002).

Unfortunately, problems experienced with molecular techniques that forced a change in methods between sample sets preclude making comparisons among sample sets. It would have been interesting to look at temporal differences or similarities in the microbial assemblages. However, based on the lack of patterns across the spatial scale and the fact that the samples could not be collected from exactly the same locations, similar heterogeneity would have probably been observed over time also. The cluster analysis did, however, show slight grouping of some samples derived from similar depths. This suggests that hyporheic microbial assemblages are more likely to be structured by changes in environmental and (or) biological parameters that vary among depths below streambed rather than by those that vary between horizontal locations within the riffle.

Similar to the results of the cluster analysis, microbial OTU richness did not appear to vary significantly between zones or among depths. The only exception was after the manipulation, when microbial OTU richness was significantly lower in the originally downwelling site than in the upwelling site. The opposite was expected, since the upstream site was more influenced by surface water and thus most likely received a greater diversity and (or) amount of labile food in the form of POM and DOC than the downstream site, which was dominated by groundwater. Groundwater-dominated areas, such as the deeper sediments, have been shown to contain less complex microbial assemblages than near-surface areas (Chandler et al. 1997).

The proportion of OTUs that was unique to a single site or depth was largest in the downwelling zone and at 40 cm depth before and during the flow manipulation. The larger number of unique OTUs in the downwelling zone versus the upwelling zone may have resulted from the larger diversity of food for hyporheic microbes carried by infiltrating stream water. The larger proportion of OTUs that were unique to an intermediate depth for these two sampling periods may have resulted from a greater mixing of groundwater and surface water at that depth compared with the other depths. Microorganisms are known to concentrate at interfaces, where nutrient and energy levels tend to fluctuate (Ash et al. 2002), and the boundary between hyporheic water and groundwater may be one such region. After the manipulation, the upwelling area had a greater number of OTUs. This followed the trend of microbial richness and once again could not be sufficiently explained, since the opposite was expected. The number of unique OTUs decreased with depth for this sampling period. This may be because the hyporheic zone – groundwater boundary had moved closer to the streambed, which is the general trend during this season (Fraser and Williams 1998).

Influence of physicochemistry in structuring microbial assemblages

There was a lack of correlation between microbial richness and all the physicochemical and biological parameters. The only exception to this occurred during the flow-manipulation period, when microbial richness was positively correlated with temperature and VHG. This may have been primarily due to the relatively large increase in temperature and VHG caused by the S1 manipulation structure (where the downwelling area was converted to upwelling). The observed effect on the diversity of the microbial assemblage within this manipulated area could have been caused by a significant alteration to temperature, which may have encouraged the growth of taxa that are normally numerically less dominant under natural conditions (e.g., nitrifiers; Storey et al. 1999). These taxa would have been underestimated in the other hyporheic areas sampled at the same time, since the techniques used in this study are subject to biases common to all molecular methods (e.g., DNA extraction and PCR). The degree of change in these parameters, although within normal seasonal and spatial fluctuations, may have been larger than is natural for this time of the year and may have forced a significant change in the microbial assemblage. Additionally, the conversion of an area of streambed from a faster flowing to a slower flowing environment could have had a significant influence on the assemblage structure. However, it is very difficult to devise an in-situ method for subsurface flow manipulation that does not affect other parameters in the environment.

The CCA results, although not significant, most likely because of the large number of species included in the analysis, did show some interaction trends between microbial assemblages and environmental variables. Depth did not play an important role before the manipulation because many of the 60 cm depth samples contained too little DNA

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to be PCR-amplified. However depth did appear to be a gradient with which bacterial assemblages were associated (mostly negatively) during and after the flow manipulation. Sediment size fraction <250 µm, with which microbes were mostly negatively associated, appeared to be a structuring variable in both pre- and post-manipulation sample sets. It thus appears that microbial assemblages tended to be more associated with larger sediment fractions and shallower sediments.

The other variables that were correlated with the CCA microbial axes were POM before the manipulation, temperature during all sampling periods, DO during the manipulation, and pH during and after the manipulation. POM appeared to be a predictor of bacterial abundance in the hyporheic zone, most likely because microbes in hyporheic sediments are primarily associated with organic particles or biofilms that coat inorganic particles (Findlay and Sobczak 2000). However, since bacteria can form a substantial fraction of the organic carbon present (Bostrom 1988), bacteria and sediment organic matter may be autocorrelated to some extent. The relationship between bacteria and POM must therefore be interpreted with caution. The quality of organic matter, a factor not measured here, may have an important effect on hyporheic microorganisms, since compositional differences in source waters can influence bioavailability to sediment bacteria (Findlay and Sobczak 2000). For example, groundwater-derived DOC tends to be less labile than that derived from surface water. This may account for the lack of correlation between DOC and the bacterial assemblage in this study. Temperature tends to be positively correlated with sediment bacterial biomass and abundance (Schallenberg and Kalff 1993). Bacteria may respond to temperature changes only at the assemblage level, with individual species being highly adapted to a specific temperature. In this case, the total bacterial abundance may remain the same, while numbers within various populations change. A negative effect of temperature on bacterial abundance noted in other studies could be due to its collinearity with depth (Schallenberg and Kalff 1993). DO was also a structuring variable, possibly because aerobic metabolic processes are more efficient than anaerobic ones (Storey et al. 1999), which may have caused a numerical, and thus a PCR bias towards aerobic microbes. This is further supported by the groups identified with RDP, as well as the relatively high average OTU richness in the S1 manipulation site, where there was a significant reduction in DO during the flow-manipulation portion of this study. As previously mentioned, pH is considered to be a predictor of assemblage respiration because it decreases with increasing CO_2 (Brunke and Gonser 1997). It was therefore not surprising to see that microbial assemblages were associated with this parameter.

The generally low structuring influence of the measured environmental and biological variables on the microbial assemblages may be an indication that bacterial-assemblage composition was influenced by factors other than those measured in this study. One such factor could be grazing by bacteriovorous protozoans, which have been shown to be an important link in the flow of materials and energy between bacteria and higher trophic levels in other systems (the "microbial loop"; Azam et al. 1983). Heterotrophic flagellates, for example, have been shown to be important grazers on bacteria in the water column (Azam et al. 1983). However, since very little is known about the composition, abundance, and activity of protozoans in the hyporheic zone, one can only speculate as to what kind of a structuring influence this trophic level may have, based on information from other systems. Sleigh et al. (1992) reported that protozoan grazing removed 20% of bacterial production in an English chalk stream. Bott and Kaplan (1990) showed, through grazing studies at 20 °C, that ciliates and flagellates could consume 80%–183% of estimated annual surface sediment bacterial production in White Clay Creek, Pennsylvania, USA. Another factor that may influence bacterial abundance and assemblage composition is virus-induced mortality. Spatially complex subsurface sediments could represent more of an obstacle to the movement of protozoans than to the diffusion of small viruses (Torsvik et al. 2002). The influence of protozoans and viruses may also depend on the nutrient status of the system studied, and could have significantly different effects on bacterial-assemblage composition. For example, Weinbauer and Peduzzi (1995) predicted that heterotrophic nanoflagellates would be expected to be more important as sources of bacterial mortality in oligotrophic systems, whereas viruses should be more important in nutrient-rich environments. Size-selective grazing by protozoans could influence how biomass is distributed into functional groups, and infection by host-specific viruses could determine how functional groups are divided into species (Torsvik et al. 2002).

In conclusion, despite the fact that the manipulation portion of this study was somewhat compromised, largely as a result of in-situ phenomena, the latter contributed useful and original findings to this exploratory study of hyporheic microbial assemblages. Foremost among these was the observed lack of change induced in bacterial OTU richness over a range of physicochemical conditions during the pre- and post-manipulation phases, but the positive correlation with water temperature when this single variable was pressed (albeit not by design) during the manipulation. Further, the persistence of microbial-assemblage heterogeneity throughout the three sampling periods and throughout the flow manipulation is significant. The finding that interstitial riverine bacterial assemblages were influenced by sediment depth and particle size underlines the importance of the scale at which such studies should be done.

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Bayesian removal estimation of a population size under unequal catchability

Samu Mäntyniemi, Atso Romakkaniemi, and Elja Arjas

Abstract: We introduce a Bayesian probability model for the estimation of the size of an animal population from removal data. The model is based on the assumption that in the removal sampling, catchability may vary between individuals, which appears to be necessary for a realistic description of many biological populations. Heterogeneous catchability among individuals leads to a situation where the mean catchability in the population gradually decreases as the number of removals increases. Under this assumption, the model can be fitted to any removal data, i.e., there are no limitations regarding the total catch, the number of removals, or the decline of the catch. Using a published data set from removal experiments of a known population size, the model is shown to be able to estimate the population size appropriately in all cases considered. It is also shown that regardless of the statistical approach, a model that assumes equal catchability of individuals generally leads to an underestimation of the population. The example indicates that if there is only vague prior information about the variation of catchability among individuals, a very high number of successive removals may be needed to correctly estimate the population size.

Résumé : Nous utilisons un modèle de probabilité bayésien pour estimer la taille d'une population animale à partir de données de retrait. Le modèle se base sur la présupposition que, dans un échantillonnage par retraits, la capturabilité peut varier d'un individu à un autre, ce qui semble nécessaire pour décrire de façon réaliste de nombreuses populations biologiques. Une capturabilité individuelle hétérogène engendre une situation dans laquelle la capturabilité moyenne de la population décroît graduellement à mesure que le nombre de retraits augmente. Avec cette présupposition, le modèle s'ajuste à n'importe quelle série de données de retrait; il n'y a donc pas de limites en ce qui a trait à la capture totale, au nombre de retraits ou au déclin de la récolte. L'utilisation de données de retrait publiées obtenues sur une population de taille connue montre que le modèle est capable d'estimer la taille de la population de façon adéquate dans tous les cas étudiés. De plus, indépendamment de l'approche statistique utilisée, un modèle qui présuppose une capturabilité individuelle uniforme sous-estime la population. L'exemple montre que, s'il n'existe que des informations préalables vagues sur la capturabilité individuelle, il faudra peut-être un nombre très élevé de retraits successifs pour pouvoir estimer correctement la taille de la population.

[Traduit par la Rédaction]

Introduction

In addition to mark–recapture, removal sampling is commonly used for the estimation of animal population size (Otis et al. 1978; Bohlin et al. 1990; Hammill and Smith 1990). In removal sampling, individuals are removed from the population in successive trials without replacement. Statistical analysis of removal data has traditionally been based on the assumption of equally catchable individuals in the population (Schwarz and Seber 1999). Early methods based on this assumption are not able to deal with nondeclining catches in successive trials (Moran 1951; Zippin 1956; Bohlin et al. 1989). However, this restriction is to some extent relaxed by the method of Carle and Strub (1978).

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Most of the statistical methods for removal data are developed in the frequentist framework. The output of the analysis comes then in a form of a value of an estimator and its estimated standard error, the latter describing the variability of the values of the estimator in hypothetical repetitions of the removal sampling. Effectively, these methods provide formal inference about future data under a hypothesis (Pierce 1973). However, usually the goal is the opposite, i.e., to make inferences about particular hypotheses of interest based on the data that we have actually observed. In addition, there may also be relevant background information, which should be properly incorporated into the analysis. The Bayesian approach to statistical inference provides systematic methods for reaching such goals (O'Hagan 1994).

Wyatt (2002) introduced a Bayesian model for removal data, assuming equal catchability of individuals. He also adopted a hierarchical approach to modeling that allows one to gather information about catchabilities and population densities from different sampling sites. In general, this results in an improved precision of the site-specific population estimates.

Electrofishing is widely used as a method for producing removal data. The method is commonly used to estimate the size of an individual fish population, but it has also been applied to assess fish species assemblages and stream inverte-

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brate populations (Hickley 1990; Pusey et al. 1998; Taylor et al. 2001). When electrofishing equipment is used to remove individuals from a fish population, sampling is often found to be size selective (e.g., Borgstrøm and Skaala 1993; Anderson 1995; Dolan and Miranda 2003). Selectivity means that the catchabilities of different individuals are generally not equal. Unequal true catchabilities tend to bias methods based on equal catchabilities downwards and underestimate the true population size (Zippin 1956). Electrofishing experiments with a known population size have revealed that such underestimation occurs in practice when methods assuming equal catchability are applied (Mahon 1980; Bohlin and Cowx 1990; Riley et al. 1993). In most such cases, the removed fraction of the remaining population declines in successive removals, indicating that the catchability declines during the experiment. This has been suggested to arise from unequal but constant catchabilities of individuals (Bohlin and Cowx 1990) or from a change of the equal catchability during the experiment (Libosvársky 1990) or a combination of both. Whatever the reason for the phenomenon may be, it invalidates the use of simple models assuming equal catchability.

Various attempts to formulate statistical models for the case of unequal catchability have been made in the past. A proposition of Schnute (1983) was to assume that there are two catchabilities, one for the first removal and another for later removals. Schnute (1983) also proposed a three-parameter function for the decline of the catchability, and a two-parameter function has been suggested by Scruton and Gibson (1995). In contrast, Otis et al. (1978) and Chao and Chang (1996) discussed a general model where the catchability is assumed to be randomly distributed in the population.

In this paper, we present a Bayesian model for removal data, which is based on the assumption of randomly distributed individual catchabilities in the population. By using a published removal data set of a known population size, we demonstrate that the model provides plausible estimates of the population size, whereas a special case of this model (assuming equal catchability) underestimates the true population size. This work can be seen as an extension of the Bayesian removal model introduced by Wyatt (2002).

Materials and methods

Model structure

We consider a population of an unknown size N_0 and attempt to make inferences about N_0 using removal sampling. We assume that the size N_0 of the population does not change during the experiment (i.e., there is no immigration, emigration, reproduction, or mortality). Let us then assume that an individual member ($k = 1,\ldots, N_0$) of the population can be characterized by a catchability parameter p_k, which is considered as an independent random draw from a (prior) probability density function $f(p_k|\mu, \sigma)$ with population-specific hyperparameters, mean μ, and standard deviation σ. Now, conditionally on the catchability p_k, let z_k be a Bernoulli-distributed indicator variable that gets value 1 if individual k is captured in the first removal and value 0 otherwise. Then the probability of obtaining z_k given p_k is $p_k^{z_k}(1 - p_k)^{1-z_k}$. A straightforward computation leads to the following simple expression for the conditional distribution of z_k given μ and σ:

$$(1)\qquad p(z_k|\mu,\sigma) = \int_0^1 p_k^{z_k}(1-p_k)^{1-z_k} f(p_k|\mu,\sigma)\,dp_k = \mu^{z_k}(1-\mu)^{1-z_k}$$

Now, all z_k values are assumed to be conditionally independent given the common hyperparameter μ. This reflects the idea that individuals respond independently to the removal. Thus, the number (x_1) of individuals captured in the first removal is binomially distributed given the number of individuals N_0 and the mean catchability μ:

$$(2)\qquad x_1 = \sum_{k=1}^{N_0} z_k | N_0, \mu \sim \mathrm{Bin}(N_0, \mu)$$

After the first removal, $N_0 - x_1 = N_1$ individuals still remain in the population. The distribution of catchabilities in the remaining population has also changed from the initial distribution $f(p_k|\mu, \sigma)$ because typically, individuals with the highest catchabilities have been removed. This can be seen by using the results of eq. 1 and employing standard probability calculus (Bayes' formula):

$$(3)\qquad f(p_k|z_k = 0,\mu,\sigma) = \frac{P(z_k = 0|p_k) f(p_k|\mu,\sigma)}{P(z_k = 0|\mu,\sigma)} = \frac{p_k^0(1-p_k)^1 f(p_k|\mu,\sigma)}{1-\mu} \propto f(p_k|\mu,\sigma)(1-p_k)$$

Therefore, the probability density of catchability of individuals in the population that remain after the first removal is proportional to $f(p_k|\mu, \sigma)(1 - p_k)$. More generally, the distribution of catchability before the jth removal is proportional to $f(p_k|\mu, \sigma)(1 - p_k)^{j-1}$. It is easy to see from eqs. 1 and 2 that only the expectation of the distribution of catchability is needed to model the number of individuals captured in each removal. By assuming that an individual catchability is initially distributed according to a Beta(α, β) (prior) distribution, it is ensured that, conditionally on parameters α and β, the expectation of the distribution of the catchability p_k for each removal j is always available in a closed form expression. This can be seen from the density function of the Beta(α, β) distribution:

$$(4)\qquad f(p_k|\alpha,\beta) = \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} p_k^{\alpha-1}(1-p_k)^{\beta-1}$$

for which the mean is $\alpha/(\alpha + \beta)$. Multiplying density (eq. 4) by $(1 - p_k)^{j-1}$ yields a Beta(α, $\beta + j - 1$) density function for which the mean is $q_j = \alpha/(\alpha + \beta + j - 1)$.

Unfortunately, the parameterization of a beta distribution in terms of its mean μ and standard deviation σ turns out to be difficult: they cannot be assessed independently because the largest possible value of σ depends on the mean ($\sigma < \sqrt{\mu(1-\mu)}$). Therefore, we choose a different parameterization by introducing a new parameter $\eta = (\mu(1 - \mu) - \sigma^2)/\sigma^2 = \alpha + \beta$, which then serves as a measure of relative

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variation. This parameterization also simplifies the parameter transformation

$$\alpha = \mu\eta \qquad \beta = (1-\mu)\eta \tag{5}$$

Thus, by assuming that the catchabilities are a sample from a beta distribution, and knowing that only the mean catchability q_j in the remaining population after $j-1$ removals is needed to model the successive catches, we can write the model in the form

$$x_j | N_j, q_j \sim \mathrm{Bin}(N_j, q_j) \qquad N_j = N_{j-1} - x_{j-1} \qquad q_j = \mu\frac{\eta}{\eta + j - 1},\ j = 1,\ldots,k \qquad x_0 = 0 \tag{6}$$

It can be seen from eq. 6 that the rate at which the mean catchability declines between consecutive removals depends on the variation of the catchability in the initial population (Fig. 1). A similar kind of change in the distribution of catchability has also been described by Bohlin and Cowx (1990).

From eq. 6, one can see that in this model, there are three parameters ($\Theta = (N_0, \mu, \eta)$) to be assessed. In the Bayesian approach, prior beliefs about these parameters are expressed in terms of prior probability distributions. Assigning a unit probability to a high value of η, say 20 000, yields a model that effectively is equal to the model proposed by Wyatt (2002).

Parameter estimation

In the Bayesian approach, parameter estimation is the process of updating the prior probability distributions of parameters of interest to posterior probability distributions by using observed data. According to the Bayes' theorem, the joint posterior probability density of model parameters ($\Theta = (N_0, \mu, \eta)$), given the observed data ($X = (x_j)$), is obtained as

$$p(\Theta|X) = \frac{P(X|\Theta)p(\Theta)}{\int p(X|\Theta)p(\Theta)\,d\Theta} \propto p(X|\Theta)p(\Theta) \tag{7}$$

Here, $p(\Theta)$ denotes the prior density of model parameters, and the term $p(X|\Theta)$ denotes the probability density of data given the parameters (the likelihood of data), which we have actually completely defined in eq. 6. In fact, Bayes' theorem was already in use in eq. 3.

Because the integral in the denominator of eq. 7 is analytically intractable, numerical integration methods are needed. Our computational approach is to use the Markov chain Monte Carlo simulation (Gilks et al. 1995) to draw samples from the posterior distributions of model parameters by implementing the simulation in WinBUGS software (Spiegelhalter et al. 2003). The use of this software in the context of fisheries science has been demonstrated and discussed, for example, by Meyer and Millar (1999) and Millar and Meyer (2000), and especially in the context of removal sampling by Wyatt (2003).

Example

To demonstrate the performance of our model, we apply it to a data set with a known population size. To illustrate the influence of different model assumptions, we also fit a restricted version of the model, assuming equal cathcability of individuals. The model allowing unequal cathcability is denoted by M_u and the model allowing only equal catchability by M_e.

Bohlin and Sundström (1977) stocked 150 juvenile brown trout (*Salmo trutta*) to three closed stream sections and conducted up to 20 removals at each sampling site i (Table 1). A detailed description of the study design is provided in their article. The large number of removals gives us an opportunity to study how increasing the number of removals affects the posterior distributions of the population size $N_{0,i}$ and parameters μ_i and η_i. Because Bohlin and Sundström (1977) did not mention the possibility that there could be mortality and (or) emigration, we simply assume that no mortality and (or) emigration took place. Total catches of each site after 20 removals were 141, 144, and 146, indicating that mortality and (or) emigration during the experiment were very low, if not zero.

We assign the following prior distributions for the parameters used in model M_u:

$$\log(N_{0,i}) \sim \mathrm{Unif}(0,10) \qquad \log(\eta_i) \sim \mathrm{Unif}(0,10) \qquad \mu_i \sim \mathrm{Beta}(1.1,1.1) \tag{8}$$

These priors are vague, effectively saying that we have little prior information about the model parameters and that we pretend not to know the true population size. Similar prior distributions are also used in model M_e, except that the value of $\log(\eta)$ is assumed to be 10, i.e., it is assumed that there is negligible variation in catchability between different individuals in the population. Thus, models M_u and M_e reflect different prior beliefs about the variation in catchability: model M_u represents the view that the catchability can be highly variable with $\log(\eta)$ being anywhere between 0 and 10, whereas model M_e reflects the idea that we are sure that the catchability is equal (unit probability on $\log(\eta) = 10$).

Posterior distributions for the model parameters were calculated by using both models five times with the observations from the first 4, 8, 12, 16, and 20 removals, respectively. To see how well models M_u and M_e can estimate the differences in the population sizes between sampling sites, we calculated posterior distributions for all pairwise differences. WinBUGS code used for the implementation of the Markov chain Monte Carlo simulation can be found in Appendix A.

According to model M_u, the posterior distributions for each sampling site obtained by using four removals favored population sizes smaller than the true value (Fig. 2). A similar pattern can also be seen after eight removals, but now, higher probabilities are assigned to values close to the true population size. After 12 or more removals, the locations of posterior distributions did not change markedly, only the precision of the distribution increased as well as the probability assigned to the true population size. All posterior distributions of pairwise differences in abundance between

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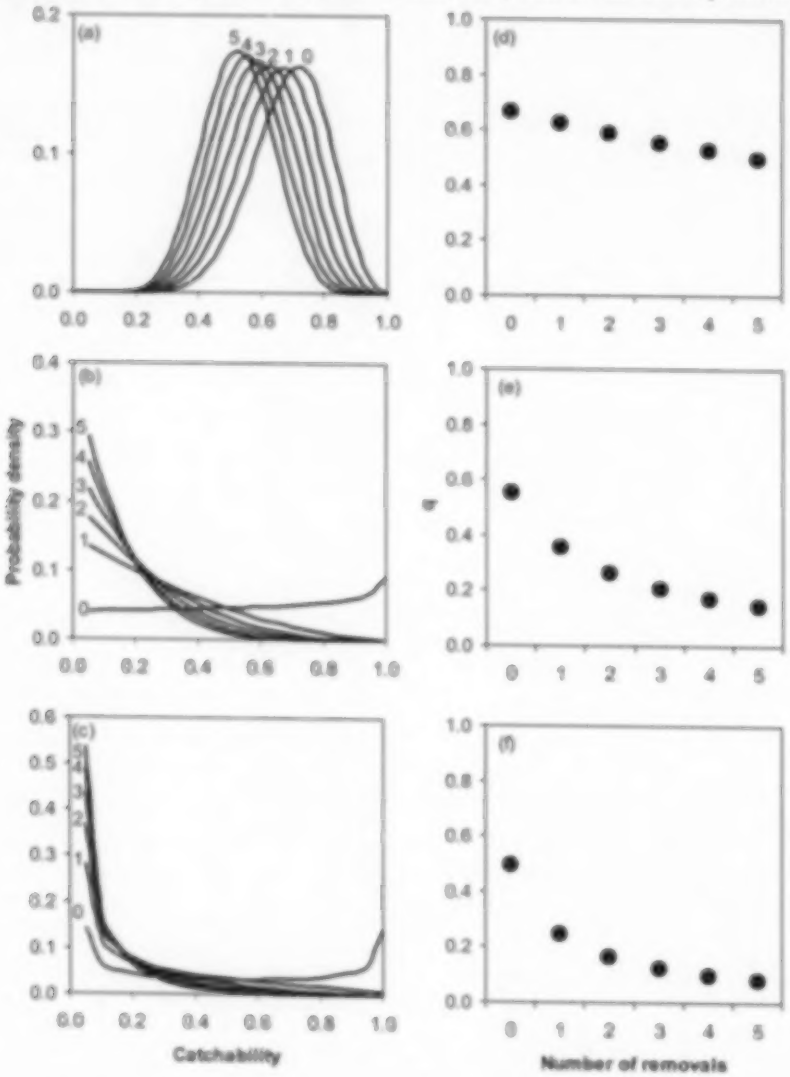
Fig. 1. (*a–c*) Evolution of the distribution of catchability in the remaining population after different numbers of removals (the number of removals is marked next to the corresponding probability density) and (*d–f*) the decline of the mean catchability (q) as a function of the number of removals taken. In all cases, the initial distribution of catchability is a beta distribution. The parameter values $\mu = 0.66$ and $\eta = 15$ are used in Figs. 1*a* and 1*d*, $\mu = 0.55$ and $\eta = 1.8$ in Figs. 1*b* and 1*e*, and $\mu = 0.5$ and $\eta = 1$ in Figs. 1*c* and 1*f*. Parameter μ is the mean catchability in the initial population (no removals taken) and η describes the relative precision of the distribution.



sampling sites also included the true value in their 95% posterior probability intervals (Fig. 3).

The reason for the apparent underestimation of the population size when using a small number of removals and model M_o can be seen from the posterior distributions of $\log(\eta)$ and μ. After four removals, the posterior distributions of $\log(\eta)$ were quite close to their prior distributions, which gave relatively high probabilities to large values of $\log(\eta)$, thus favoring only a small decline in mean catchability between consecutive removals. In other words, data from four removals contained so little information that the prior distribution of $\log(\eta)$ still had a dominant influence on the posterior distributions. As the number of removals used in the analysis increased, the influence of the prior distribution decreased.

Because the inference about population size appears to be quite sensitive to the choice of the prior distribution for $\log(\eta)$ when only a few removals are conducted, we wanted to study how much information about $\log(\eta)$ was contained in the complete data. For this purpose, we calculated the posterior distribution of $\log(\eta)$ not only by using the observed data from all 20 removals but also by using the information of the true population size (simply by treating the true population size as an observation instead of having prior distribution for N). As would be expected, these posterior distributions (Table 2) are quite similar to the posterior dis-

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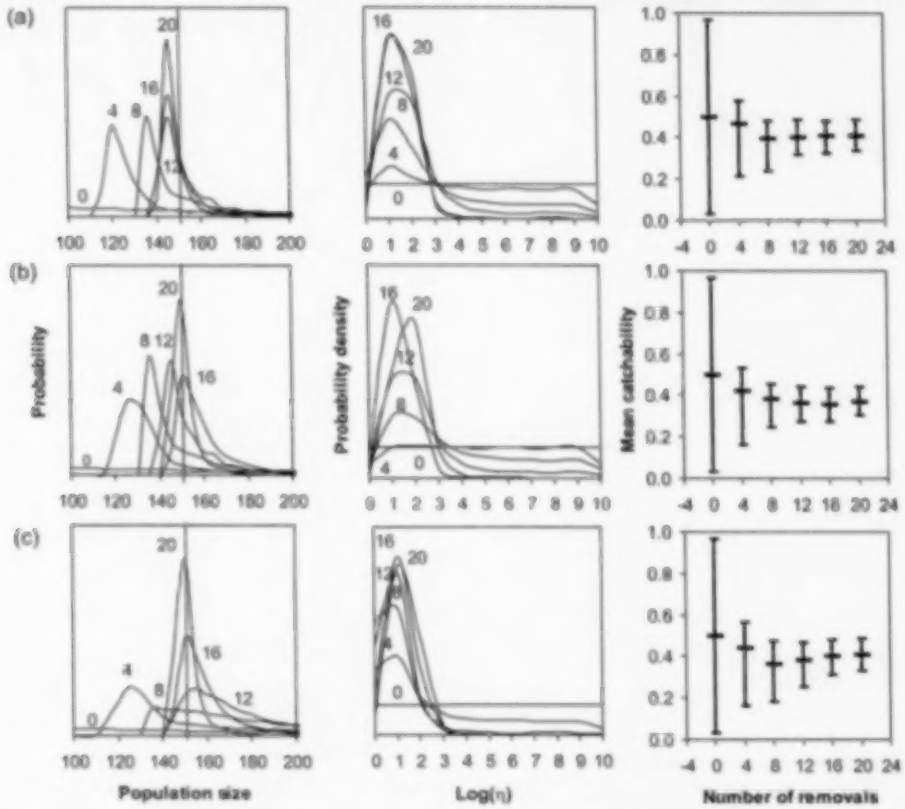
Table 1. Removal data from the experiment of Bohlin and Sundström (1977) (modified from their article).

Site	Successive catches																			
a	62	26	17	8	9	6	4	1	2	1	1	1	1	1	0	1	0	0	1	0
b	57	28	22	8	3	10	2	3	5	2	0	1	2	0	2	1	0	0	0	0
c	66	25	13	12	9	5	4	3	2	2	2	1	1	0	1	0	1	0	0	0

Fig. 2. Posterior distributions of population size N and dispersion parameter log(η) and quantiles (2.5%, 50%, and 97.5%) of the posterior distributions of the mean catchability μ in three sampling sites (*a* (site a), *b* (site b), and *c* (site c)) calculated by a model assuming unequal catchabilities of individuals. The number of removals used to calculate each posterior distribution of N and log(η) is indicated next to the corresponding probability distribution. Probabilities and probability densities are presented in relative scale.



tributions calculated using only the 20 removals (Fig. 2) but are more precise.

Results obtained by model M_c show that the precision of the posterior distributions is very high, but the estimation of the true population size is not successful even when using 20 removals because the posterior distributions are systematically concentrated on values that are too low (Fig. 4). With all 20 removals, model M_c produces very tight posterior distributions for the differences in population sizes between sampling sites (Fig. 3). But even then, two out of three 95% probability intervals failed to include the true value.

The results obtained by Bohlin and Sundström (1977) are not directly comparable with ours because of their fundamentally different approach to statistical inference. However, their frequentist approach assumes constant catchability among individuals, as, in effect, does our model M_c. From the 95% confidence intervals based on two, three, and four removals, only one included the true population size (site b, three removals) and the remaining eight intervals suggested values lower than the true population size (Bohlin and Sundström 1977).

Discussion

Properties of the model

A Bayesian model for the estimation of population size from removal data and assuming equal catchability was recently developed by Wyatt (2002). Here, we have taken a step further towards more realistic model assumptions and developed a Bayesian model (M_u) that allows heterogeneity in catchability in the population. Our example demonstrated how model M_u can provide reasonable estimates of the true

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Fig. 3. Quantiles (2.5%, 50%, and 97.5%) of the posterior distributions of the difference between population sizes in three sampling sites (a, b, and c) calculated by a model assuming (*a*) unequal capture probabilities and (*b*) equal capture probabilities. The true value for the difference between all sites is zero.

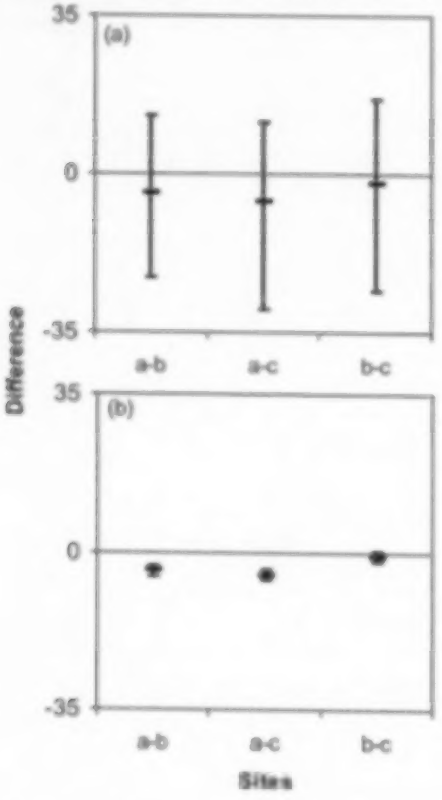


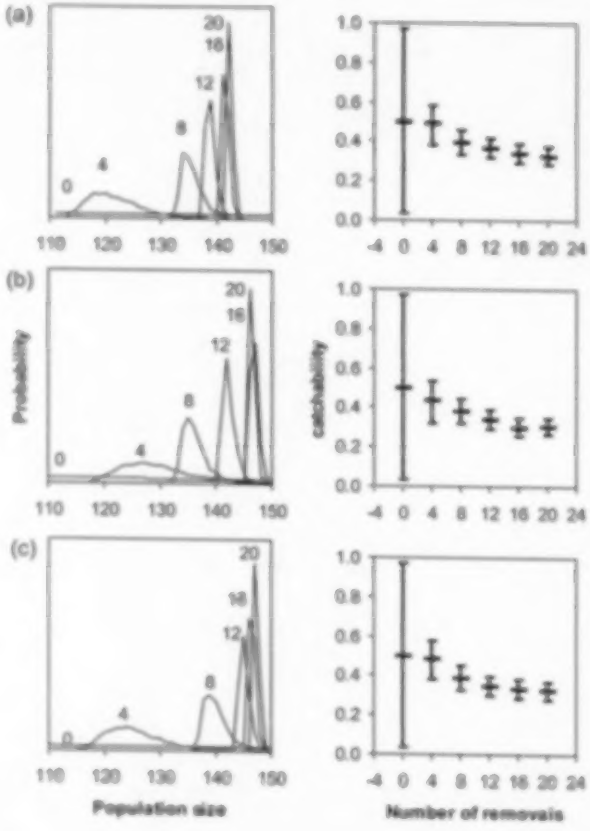
Table 2. Statistics of posterior distributions of dispersion parameter log(η) obtained by using 20 removals (Table 1) and also the known population size of 150 from three sampling sites as observed data.

	log(η)				
Site	Mean	SD	2.5%	50%	97.5%
a	1.124	0.2645	0.6475	1.108	1.685
b	1.702	0.3491	1.094	1.682	2.484
c	1.561	0.3491	0.9223	1.543	2.301

population size in cases in which the models assuming equal catchability fail.

The Bayesian model M_h and its special case M_0 are suitable for a probabilistic assessment of the uncertainty about the population size when based on observed removal data and background information. They can also tolerate all kinds of catch vectors, that is, catches in the successive removals do not need to decline, as in many earlier removal methods. The number of removals conducted can vary from one to any attainable number, and also, the catch per removal can vary from zero to thousands, without violating the mathematical structure of the model or ruining the numerical computations needed in the estimation. The model proposed by Wyatt (2002) has these same advantages over corresponding frequentist removal methods but does not allow for unequal catchability.

Fig. 4. Posterior distributions of the population size N (left panels) and posterior quantiles (2.5%, 50%, and 97.5%) of the catchability p (right panels) in three sampling sites (*a* (site a), *b* (site b), and *c* (site c)) calculated by a model assuming equal catchability of individuals. The number of removals used to calculate each posterior distribution of N is indicated next to the corresponding probability distribution. Posterior probabilities are presented in relative scale.



It has been emphasized in many previous papers about removal sampling that models assuming unequal catchabilities can be used only if a large number of removals are conducted (e.g., Schnute 1983; Wyatt 2002, 2003). This idea probably has its roots in the theory of maximum likelihood estimation, where identifiability of parameters is an important issue, whereas this is less central in Bayesian inference. According to the Bayesian approach, the assumption about the catchability should be based on expert knowledge about the behavior of animals and on knowledge about the sampling method, assessed before obtaining removal data and before knowing the number of removals.

If there is uncertainty about the equality of individual catchabilities, this uncertainty should be brought into the Bayesian analysis in the form of prior probabilities. Ob-

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served data will then update the beliefs about the equality of the catchability. The prior that we gave for log(η) in the model M_u serves as an example of a high degree of prior uncertainty in this respect, i.e., all parameter values from highly variable individual catchabilities to very equal catchabilities were regarded as being possible. It was partly due to this high prior uncertainty that we pretended to have in our example that many data (many removals) were needed to produce firm posterior beliefs. However, in the case of electrofishing in rivers, for example, there is firm evidence in the literature that the catchabilities are not equal, and thus, unless some case-specific background information justifies an assumption of equal catchabilities, very low prior probabilities should be assigned to high values of log(η).

Even though model M_c is a special case of model M_u, there might be situations in which regarding model M_c as an alternative to model M_u might have some particular value. For example, if catchabilities really were practically equal, model M_u would waste the information contained in the data and use it for dragging the posterior distribution of log(η) to support values close to 10. In such a case, more precise and accurate estimates would be obtained by using model M_c. However, it is difficult to know exactly which model to use. Such an uncertainty can be accounted for by adopting an approach termed Bayesian model averaging (Hoeting et al. 1999). In Bayesian model averaging, all alternative models are assigned prior probabilities, and observed data are used to come up with posterior probabilities for all models. Posterior distribution from Bayesian model averaging is a weighted mixture of posterior distributions from individual models in which posterior probabilities of the models are used as weights. Bayesian model averaging can be implemented within the Markov chain Monte Carlo simulation by using the method of Carlin and Chib (1995), for example.

Violating the assumptions of model M_u

Although derived from a specific assumption, model M_u can also be expected to provide reasonable estimates in other cases in which the mean catchability declines between removals. The mean catchability will decline under the following circumstances. (*i*) Each individual has its own catchability, which remains constant between removals. For example, individuals with different physical properties and (or) habitats keep their positions and do not learn how to avoid removal. This is the underlying assumption of model M_u. (*ii*) All individuals in the population have equal catchability, which decreases between removals. For example, individuals not caught in one removal learn how to avoid being caught in the next removal. (*iii*) Each individual has its own catchability, which decreases between removals. For example, individuals have different habitats or physical properties and they learn how to avoid removal. (*iv*) Each individual has its own mean catchability around which the catchability varies randomly between removals. For example, individuals with different physical properties change their habitats between removals. (*v*) Each individual has its own mean catchability around which the catchability varies randomly between removals and the mean catchability decreases between removals. For example, individuals with different physical properties change their habitats between removals and learn how to avoid removal.

We have included above the possibility that individuals may learn to avoid removal (or otherwise change their behavior with similar effect on catchability) because several authors have suggested that this might be the case in electrofishing (Cross and Stott 1975; Bohlin and Sundström 1977; Libosvárský 1990). It should be noted that if all individuals in the population have the same mean catchability around which their catchabilities vary between removals, then model M_u can be used because the mean catchability does not decline between removals.

Our model is based on the assumption that individuals behave independently during the removal process. This assumption is violated if individuals are schooling. Schooling behavior increases the probabilities of high and low catches compared with the binomial distribution, thus invalidating the use of models using binomial distribution (also see Bohlin et al. (1989) and Wang and Loneragan (1996)). Catches of schooling species can be modeled more realistically by, for example, using the beta-binomial distribution (Mäntyniemi and Romakkaniemi 2002). Another common social interaction is territorial behavior. This form of dependence between individuals is unlikely to harmfully influence the performance of our model, unless it leads to an increase in the mean catchability during the removal process.

A further assumption in our model, made for mathematical convenience, was that the catchability is beta-distributed in the initial population. The beta distribution is very flexible and can represent various uni- and bi-modal shapes. Bimodal distributions that can be presented by a beta distribution have modes at 0 and 1. Other types of multimodality cannot be well captured by a beta distribution.

If the catchability is multimodally distributed in the initial population, model M_u may not be able to describe the decline in the mean catchability well enough. To avoid multimodal distributions of catchability, the population to be estimated should be divided to subgroups that are believed to have unimodally distributed catchabilities. In the case of electrofishing in streams, fish in the same sampling site should be classified according to their species and separable size groups. Because larger individuals should be generally easier to catch by electrofishing than smaller ones (Dolan and Miranda 2003), the mean catchabilities could be ordered according to size groups within a sampling site.

The performance of model M_u under various violations of its assumptions could be studied further by using simulated data. Simulated data could also be used to explore the effects of increasing or decreasing the number of removals under different conditions and to conduct cost efficiency analysis for choosing the best sampling design. Such an analysis could also compare removal methods with alternative ways of measuring the population size, like mark–recapture. However, only methods based on similar assumptions should be compared. Our removal method presented could be compared with a Bayesian mark–recapture model assuming heterogeneous catchability and having comparable prior distributions, but so far, such methods have not been developed. However, a frequentist literature for unequal catchability within mark–recapture design does exist (e.g., Lee and Chao 1994; Anderson 1995; Coull and Agresti 1999).

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Lessons to learn from the illustration

Our example illustrates a few important points regarding the use of removal methods with electrofishing. First, it gives further support to the widely shared view that the assumption of equal catchability tends to lead to underestimation of the true population size. It also shows that when the assumption of equal catchability is not tenable, using methods based on such an assumption may not provide reliable indices of abundance. Instead, results obtained by model M_c give the impression that the population sizes in different sampling sites are different with a very high probability. The variation of population density between sampling sites will therefore become overestimated if the assumption of equal catchability does not hold and model M_c is nevertheless used. Also, the temporal changes in population density and species composition will be overestimated in such a situation. These results are not in line with many previous suggestions, according to which estimates from models like M_c could be used to analyze the spatial and temporal changes in the relative population density (e.g., Bohlin et al. 1989; Bohlin 1990; Bohlin and Cowx 1990). These ideas are based on the assumption that the bias would be a constant fraction of the true population size.

However, overestimation of spatial and temporal variation might be reduced to some extent by using a hierarchical model for the site-specific abundances because hierarchical modeling tends to pull the site-specific estimates toward their mean (Wyatt 2002). On the other hand, the amount of such shrinkage may not be substantial because site-specific data are likely to be quite informative when model M_c is used. These issues could be further studied by simulating data from model M_c and by producing estimates by model M_c, with and without hierarchical prior distributions for the model parameters.

A further important issue is the number of removals to be conducted at each sampling site. Electrofishing tends to be a time-consuming and laborious activity (Mitro and Zale 2000), which poses some practical limits on the possible number of removals. Although a minimum of three removals has been the standard practice in some studies (Scruton and Gibson 1995), in practice, only one to three removals are usually used (e.g., Mitro and Zale 2000; Niemelä et al. 2000; Wyatt 2002). Theoretical results from removal experiments have pointed towards the need to perform one to five removals per site (e.g., Bohlin 1990; Riley and Fausch 1992). However, these theoretical results are based on the assumption of equal catchability under which precise estimates can be achieved. Under the conditions of our example, it seems that at least eight removals might be needed when the catchability is assumed to vary between individuals and there is poor prior knowlegde about parameter values. Of course, there are many factors that influence such recommended number of removals, like (*i*) the desired precision of the posterior distribution, (*ii*) the true population size (not known precisely), (*iii*) the true mean catchability in the initial population (not known precisely), (*iv*) the true variation of catchability in the initial population (not known precisely), and (*v*) the amount of prior uncertainty about the population size and catchability parameters.

Of these, only the desired precision of the estimate and the amount of prior uncertainty can be specified in advance, and all others are uncertain quantities before and after collecting data. The knowledge about the uncertain parameters is expressed in the form of prior distributions. This means that prior distributions can be used to simulate multiple data sets with a large number of removals taken from the population. Analyzing such simulated data sets by using different numbers of removals can give some clue about the number of removals needed. If the mean catchability is high and the variation in catchability is low and there is much prior knowledge about all parameters, then the number of removals that are needed will be lower and vice versa.

As there are practical limitations regarding the number of removals possible in electrofishing, a parallel strategy to improve the precision of the population density estimates would be to use all feasible sources of information to reduce the prior uncertainty about the population size and catchability parameters. Within the Bayesian framework, this can be done by utilizing the idea of hierarchical modeling, which makes it possible to transfer information between, for example, different sampling sites. Wyatt (2002, 2003) has described the use of hierarchical modeling and the use of Geographic Information System data for improving the estimates of population density from electrofishing in streams. The model presented here could be extended to allow for simultaneous mark–recapture and removal estimation, which would under suitable circumstances improve the information content of data and thus reduce the number of removals needed. For example, individuals caught in the first removal could be marked and released to the population before continuing with further removals, as suggested by Scruton and Gibson (1995).

Because our example indicates that the prior knowledge about the variation in catchability between different individuals ($\log(\eta)$) has a dominant role in the resulting inference, it might be worthwile to study whether there are parameter values that are inherent for particular species and (or) sampling circumstances. For instance, the posterior distribution of $\log(\eta)$ could serve as a prior distribution of the parameter under conditions believed to be similar to those of Bohlin and Sundström (1977). Finding such a range of commonly applicable values for different situations would then release some of the pressure for conducting unpractically high numbers of successive removals in each sampling. Conducting specially designed removal experiments of a known population size and a large number of removals using different combinations of species and sampling circumstances would provide information about realistic values of $\log(\eta)$ and also about the influential factors in each such situation. In addition to Bohlin and Sundström (1977), Mahon (1980) and Riley et al. (1993) have also published removal data sets with known true population size, which are readily available for examination of $\log(\eta)$. Posterior distributions from these kinds of studies can either be used directly as prior distributions or be used as starting points when eliciting case-specific prior information.

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Appendix A

This appendix contains the entire WinBUGS 1.4 program required to implement the example. Note that all text after the character # is comments until the end of the line.

```
model{
for(j in 1:k){
 x[j]~dbin (q[j], n[j])
 n[j+1] <- n[j] -x[j]
 q[j] <- mu*(eta/ (eta+j-1))
 }
n[1] <- exp(u)
u~dunif (0, 10)
eta <- exp(log.eta)
log.eta~dunif (0, 10)
#log.eta <- -10 # needed only when restricting M_u to M_e
mu~dbeta(1.1, 1.1)
}
```

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Bottom-up limitation of a stream salamander in a detritus-based food web

Brent R. Johnson and J. Bruce Wallace

Abstract: The indirect effects that resources can have on higher trophic levels remain poorly understood for detritus-based ecosystems. Our objective was to examine effects of long-term terrestrial litter exclusion on a larval salamander, *Eurycea wilderae*, in a detritus-based stream. After 4 years of exclusion treatment, we conducted a mark–recapture study and analyzed gut contents of *E. wilderae* larvae in the litter exclusion reach, a reach downstream of treatment, and in a reference stream. *Eurycea wilderae* growth rate (per day), density (individuals per square metre), biomass (milligrams ash-free dry mass per square metre), and production (milligrams ash-free dry mass per square metre per year) were all significantly reduced in the litter exclusion reach. Reduced density in the treatment reach was likely due to elevated hatchling drift driven by reduced prey availability. Larvae from the treatment reach had fewer prey items per gut than larvae in the reference stream and their diet consisted of fewer copepods but more midge larvae, nematodes, and terrestrial insects. The reach downstream of treatment was intermediate between reference and litter exclusion reaches for most measured parameters, indicating residual effects of upstream treatment. Our results provide the first comprehensive evidence of bottom-up limitation of a vertebrate predator in a detritus-based ecosystem and further demonstrate the importance of the terrestrial–aquatic linkage.

Résumé : Les effets indirects possibles des ressources sur les niveaux trophiques supérieurs restent mal compris dans les écosystèmes basés sur le détritus. Notre but est d'évaluer les effets de l'exclusion de la litière terrestre pendant une longue période sur les larves de la salamandre *Eurycea wilderae* dans un cours d'eau fonctionnant à base de détritus. Après 4 années d'exclusion de la litière, nous avons complété un inventaire par marquage et recapture et analysé les contenus stomacaux des larves d'*E. wilderae* dans la zone d'exclusion, dans une zone en aval de celle-ci et dans un cours d'eau témoin. Le taux de croissance ($jour^{-1}$), la densité ($individus \cdot m^{-2}$), la biomasse (mg de masse sèche sans les cendres (AFDM)$\cdot m^{-2}$) et la production (mg AFDM$\cdot m^{-2} \cdot an^{-1}$) sont tous significativement réduits dans la zone d'exclusion de la litière. La densité réduite dans la zone expérimentale s'explique vraisemblablement par une dérive accrue des larves néonates causée par une réduction de la disponibilité des proies. Les larves de la zone expérimentale ont un nombre plus petit de proies par tube digestif que les larves du cours d'eau témoin et leur régime alimentaire contient moins de copépodes, mais plus de larves de chironomidés, de nématodes et d'insectes terrestres. La plupart des paramètres mesurés dans la zone en aval de la zone expérimentale ont des valeurs intermédiaires entre celles de la zone d'exclusion et celles de la zone témoin, ce qui indique l'existence d'effets résiduels de l'exclusion en amont. Nos résultats apportent les premières preuves détaillées de l'existence d'une limitation ascendante d'un vertébré prédateur dans un écosystème à base de détritus et ils démontrent l'importance du lien entre les milieux terrestres et aquatiques.

[Traduit par la Rédaction]

Introduction

Detritus, consisting of nonliving organic matter and associated microbes, often serves as the major fuel driving ecosystems. Most consumers rely either directly or indirectly on detritus as a food resource (e.g., Fisher and Likens 1973; Wetzel 1995). Yet, even though as much as 70–90% of all global primary production eventually enters detrital pathways (e.g., O'Neill and Reichle 1980; Pomeroy 1991), the foundations of food web theory remain grounded in the traditional grazing food chain (e.g., Hairston et al. 1960; Oksanen et al. 1981). While numerous enrichment and limitation experiments have added to our understanding of the roles of resources and consumers in structuring grazing food webs (e.g., Power 1990; Spiller and Schoener 1990; Nakano et al. 1999), manipulative experiments in detritus-based ecosystems are rare and we therefore know less about the relative strengths of top-down and bottom-up forces in shaping these systems.

Detritus-based ecosystems are fundamentally different from grazing food chains because they are typically donor controlled with the basal resource originating from outside the system. Omnivory, multiple food web links, and low interaction strengths may also be common in detrital food webs and thus prevent the simplified cascading trophic inter-

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actions inherent in traditional grazing models (Strong 1992; Polis 1994; Polis and Strong 1996). Increasing evidence suggests that all trophic levels in detritus-based ecosystems can be resource limited (Richardson 1991; Wallace et al. 1997, 1999), but few studies have focused specifically on bottom-up effects of detritus on higher trophic levels (but see Polis and Hurd 1995; Chen and Wise 1999).

In a long-term ecosystem experiment, Wallace et al. (1997, 1999) reported that detritus exclusion dramatically altered benthic community structure and resulted in the lowest secondary production reported for streams. They also found that detritus limitation reduced abundance, biomass, and production of larval salamanders, the top predators in high-gradient southern Appalachian streams. The adverse effect of the exclusion treatment on predators may be significant because stream predators can be heavily subsidized from outside the system and thus rely less on in-stream resources (Mason and MacDonald 1982; Nakano et al. 1999). The studies of Wallace et al. (1997, 1999), however, combined all salamander species rather than considering responses of individual populations and were based on benthic core samples that may not accurately assess larval salamanders owing to their mobility. Additionally, the mechanisms underlying the reductions in larval salamander populations remain unclear. Reduced abundance may be due to excessive drift of larvae from the study reach, reduced adult oviposition, or increased mortality, whereas lower secondary production can be due to reduced abundance, biomass, or individual growth rate (Benke 1984).

The purpose of this study was to comprehensively examine the effects of long-term detritus reduction on the dominant salamander *Eurycea wilderae* (Blue Ridge two-lined salamander). Our specific objectives were to assess the effects of detritus limitation on *E. wilderae* population density, biomass, individual growth rate, movement, and secondary production using a mark and recapture design. Mark–recapture studies provide more accurate information about populations than benthic sampling or mesocosm studies because they can focus on responses of free-ranging individuals over the duration of the larval period. We also analyzed *E. wilderae* diets to describe potential mechanisms underlying any population differences.

Eurycea wilderae larvae are the most abundant larvae in southern Appalachian headwaters (Bruce 1985). The larval stage lasts 1 or 2 years with hatching and metamorphosis of older larvae occurring in spring and early summer (Bruce 1982, 1988; Lugthart 1991). Larvae of these species are restricted to the stream where they feed on a wide variety of aquatic invertebrates (Caldwell and Houtcooper 1973; Burton 1976; Lugthart 1991).

Study sites and litter manipulation

This study was conducted in two perennial first-order streams at the Coweeta Hydrologic Laboratory (US Forest Service) in Macon County, North Carolina. Coweeta is a 1625-ha drainage basin in the Blue Ridge Province of the southern Appalachian Mountains. The streams are characteristic of headwaters in the region and drain forested catchments dominated by mixed hardwoods, including oaks (*Quercus* spp.), hickories (*Carya* spp.), and yellow poplar (*Liriodendron tulipifera*). Dense growths of rhododendron (*Rhododendron maximum*) shade the streams throughout the year and limit primary production. Detailed descriptions of the Coweeta basin are given in Swank and Crossley (1988).

Table 1. Physical parameters of streams draining C53 (reference), C55 (litter exclusion treatment), and C56 (downstream of treatment) at the Coweeta Hydrologic Laboratory.

Variable	C53	C55	C56
Catchment area (ha)	5.2	7.5	9.9
Elevation (m above sea level)	829	810	785
Channel length (m)	100	170	65
Wetted width (m)	0.7–1.2	1.2–1.6	1.2–1.6
Average discharge ($L \cdot s^{-1}$)[a]	1.85	2.39	2.07
Annual average temperature (°C)[b]	12.2	12.2	—
Annual degree-days[b]	4485	4512	—
Bedrock outcrop (% composition)	27	13	30
Mixed substrates (% composition)	73	87	70

Note: Elevations were measured at the gauging flumes. Wetted widths were measured during dry and wet periods. Temperature and annual degree-days were not measured for C56 and were assumed to be similar to those in C55.

[a]C53, 12-year average (1984–1996); C55, 5-year average (1992–1997); C56, 4-year average (1992–1996).

[b]C53 and C55, 12-year average (1985–1997).

The study streams drain catchments C53 (reference), C55 (litter exclusion), and C56 (downstream of exclusion treatment). A 100-m reach in C53 served as a reference to examine treatment effects. Leaf litter inputs were excluded from the first 170 m of the treatment stream reach (C55) with an overhead net canopy (2.5-cm mesh) beginning in August 1993. Riparian vegetation was left intact during canopy installation to prevent changing the natural light regime. Plastic lateral drift fences (20 cm high with 1-cm mesh) were placed along each side of the treatment reach to prevent lateral inputs of terrestrial detritus or coarse particulate organic matter. Litter was removed from the canopy once each week in autumn and when needed in other seasons. Small woody debris (<10 cm in diameter) was removed from the treatment reach in summer 1996 followed by removal of large woody debris (>10 cm in diameter) in summer 1999. As a result of these manipulations, organic matter standing crop in the treatment reach has now been reduced by approximately 95% compared with pretreatment and with the reference stream (Wallace et al. 1999). A third reach, C56, extends 65 m immediately downstream of the litter exclusion treatment, so this catchment includes that of the treatment reach (C55). This reach was included to assess how the *E. wilderae* population responds to upstream detritus exclusion. The three stream reaches compared in this study have similar physical characteristics, including catchment size, discharge, and thermal regime (Table 1).

Methods

The entire wetted areas of the three study reaches (reference, treatment, and downstream of treatment) were sampled for larval salamanders approximately every month from November 1997 through April 1999. Flagging tape was placed at 5-m intervals throughout each reach to determine capture location. Stream reaches were sampled at night, when sala-

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mander larvae are most active, using a small aquarium dip net (1-mm mesh) and headlamp. Owing to several ongoing studies in these streams, only loose cover objects (e.g., cobble, wood, and leaves) were turned over when searching for larvae to minimize stream disturbance. Captured larvae were placed in individual 20-mL plastic vials filled with stream water. Vials were labeled with the point of capture to the nearest 1 m and placed in a cooler.

In the on-site laboratory, each larva was anesthetized in Petri dishes containing 0.1% tricaine methanesulfonate (MS 222) (Beachy 1994). Snout–vent length was measured from the tip of the snout to the posterior vent margin to the nearest 0.5 mm using a dissecting microscope (12× magnification) and vernier calipers. Anesthetized larvae were then given unique marks by injecting different colors of acrylic polymers (Liquitex® brand; Binney and Smith Inc., Easton, Pennsylvania) under the skin of the tail immediately behind the hind legs (Cecil and Just 1978; Johnson and Wallace 2002). This marking procedure has proven to be an effective method for long-term marking of larval *E. wilderae* and has no adverse effects on growth or survival (Johnson and Wallace 2002). After marking, larvae were revived in stream water and released at the point of capture the following morning or evening.

Mark–recapture data were used to generate monthly population size estimates for each stream reach using the Jolly–Seber full model (Jolly 1965; Seber 1965) included in the software package POPAN-5 (Arnason et al. 1998). The Jolly–Seber model is an open population model and therefore offers greater biological realism than closed models because it allows for additions and losses within the population during the study period. Monthly population size estimates were converted to density (individuals per square metre) by dividing by mean wetted area of the study reaches over the sampling period.

We measured larval growth by two independent methods. (*i*) We first compared mean biomass of all larvae collected on each sample date after hatchlings appeared in May 1998. Biomass was calculated by converting snout–vent length to ash-free dry mass (AFDM) using the length–weight regression derived for *E. wilderae* in undisturbed Coweeta streams:

$$M = 0.0023L^{3.09}$$

where M is larval mass (milligrams AFDM) and L is length (millimetres) (Lugthart 1991). (*ii*) Larval growth rates were also calculated using mark–recapture data for the entire study period. Daily growth rates (g) were calculated for all recaptured larvae as follows:

$$g = (\ln M_t - \ln M_i)/t$$

where M_i is initial larval mass (AFDM), M_t is final larval mass (AFDM), and t is the time interval between captures in days (Romanovsky and Polischuk 1982). Only initial and final masses were used for those larvae that were recaptured on multiple sample dates.

Eurycea wilderae production was calculated for the 1998–1999 cohort using the instantaneous growth method (Waters 1977; Benke 1984). Individual growth and density estimates were based on mark–recapture results, whereas biomass (milligrams AFDM per square mertre) was obtained by multiplying the population density estimate for each sample date by the mean mass of all larvae actually captured in the stream on the same date. Mean biomass, mean daily growth rate, and time interval in the sample period were multiplied to calculate interval production. Interval production estimates were then summed to get annual production (milligrams AFDM per square metre per year), which was also habitat weighted (Lugthart and Wallace 1992) to account for the fact that larvae were not found on bedrock outcrops.

Larval *E. wilderae* were not collected for diet analysis until the mark–recapture study was completed in litter exclusion and downstream reaches. Approximately five larvae of the same cohort were collected seasonally from each stream reach beginning 21 July 1999 (summer). Other collection dates were 22 October 1999 (autumn), 12 February 2000 (winter), and 23 May 2000 (spring). Seasonal samples were subsequently combined in each stream to increase statistical power for comparisons among reaches. Larvae were collected at night and immediately placed in vials of Kahle's solution. In the laboratory, guts were removed under a dissecting microscope and their contents teased out and mounted on a slide with CMC-10 (Masters Company, Inc., Bensenville, Illinois). Insect taxa were identified to genus when possible except for chironomids, which were identified as either non-Tanypodinae or Tanypodinae. Noninsect taxa were identified to order. All prey items were measured to the nearest millimetre using an ocular micrometer. Prey biomass (AFDM) was then estimated using established length–mass or head width – mass regressions (Sample et al. 1993; Benke et al. 1999).

Percent similarities (Whittaker 1975) of larval diets were calculated because they provide a simple diet comparison among streams. However, statistical differences among stream reaches were assessed using the multiresponse permutational procedure (MRPP). MRPP is a nonparametric multivariate test that has proven useful in comparing species composition data (Biondini et al. 1985; Zimmerman et al. 1985).

Ecosystem-level experiments have an advantage over studies conducted on smaller scales because they more accurately account for environmental complexity and therefore have a level of biological realism that cannot be obtained from cage or mesocosm experiments (Carpenter et al. 1995; Schindler 1998). This is especially true for studies dealing with predators because enclosures can inhibit movement, provide unnatural prey densities (Cooper et al. 1990), and alter competitive interactions. Unfortunately, reduced statistical power is often the trade-off for realism in ecosystem studies (Hurlbert 1984; Carpenter et al. 1989; Schindler 1998).

Because we lacked the appropriate replication required for most statistical tests, we compared stream reaches in most cases by using bootstrapped 95% confidence intervals (CIs) (Effron and Tibshirani 1993). Data sets were bootstrapped by random resampling with replacement until 1000 data sets were produced. These recombined data sets were used to produce vectors of 1000 estimates for each parameter. The mean and approximate 95% CIs were then calculated for each vector of estimates. If the estimates followed a normal distribution, normal 95% CIs were used. However, if the vector of estimates did not follow a normal distribution, we ordered the estimates and used the 25th and 975th estimates

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Table 2. Larval *Eurycea wilderae* captured from reference (C53), downstream of treatment (C56), and treatment (C55) streams at the Coweeta Hydrologic Laboratory from November 1997 to April 1999.

	C53	C56	C55	Total
Total individuals captured	412	323	283	1018
Total no. of recaptures	122	104	99	325
No. of larvae recaptured on multiple dates	99	70	60	229
Percent recapture	29.6	32.2	35	31.9

as the 95% interval boundaries (Blank et al. 1999). This often results in CIs that are not symmetrical around the mean, but the intervals accurately represent variation in the data. Errors associated with annual secondary production estimates were calculated by bootstrapping all parameters for each sampling interval (individual growth rate, biomass, and time) (Morin et al. 1987; Huryn 1996, 1998). Differences between mean values were considered significant ($p < 0.05$) when 95% bootstrapped CIs did not overlap, a conservative test for differences (Zar 1996). This method of comparison, however, prevents us from definitively stating that any differences between streams are due to treatment effects alone (Hurlbert 1984).

Results

A total of 1018 *E. wilderae* larvae were captured in the three stream reaches during the study period. Larvae were recaptured up to 10 months after initial marking, a period covering the majority of the larval stage for most individuals (Bruce 1988; Lugthart 1991). The reference stream had the highest number of both initial captures and subsequent recaptures, while the litter exclusion stream yielded the fewest (Table 2) despite the fact that it has the largest wetted area of the three streams (Table 1). Recapture rate was approximately 30% in each stream.

The litter exclusion stream had the lowest population size and density of the three study stream reaches. On nine of the 13 sampling dates, population size was greater in the reference stream than in the litter exclusion reach, while the that in reach downstream of treatment was typically intermediate (Fig. 1*a*, downstream reach omitted for clarity). On certain dates, recapture frequency was not sufficient to generate standard errors associated with population size estimates, and therefore, these dates were excluded from analyses ($n = 2$ for reference and downstream reaches, $n = 3$ for litter exclusion reach). The reference stream reach had a significantly larger mean population size over the study period (153 (98–233, 95% CI)) than the litter exclusion reach (65 (48–86, 95% CI)), whereas that of the reach downstream of treatment was intermediate (96 (±32, 95% CI)) and not significantly different from that of either the reference or litter exclusion reaches (Fig 1*b*). Differences between stream reaches were even more apparent when population size estimates were converted to densities (individuals per square metre) to account for differences in stream size. Monthly larval densities in the litter exclusion reach remained well below those of reference and downstream reaches throughout the study period (Fig. 2*a*), and mean larval density in

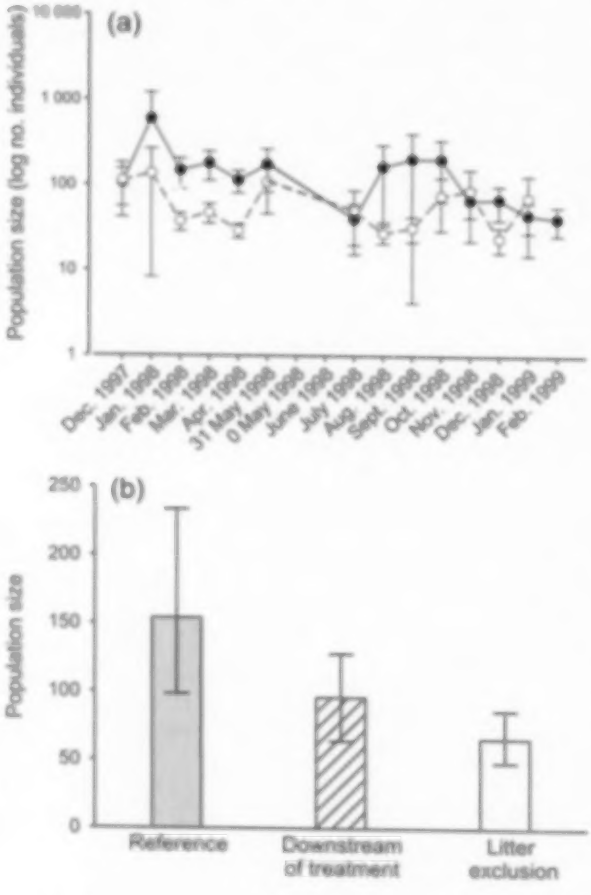

Fig. 1. Larval *Eurycea wilderae* population sizes in reference, downstream of treatment, and litter exclusion reaches from December 1997 to February 1999. (*a*) log monthly Jolly–Seber population size estimates for reference (solid circles and solid line) and litter exclusion (open circles and broken line) reaches (downstream of treatment reach omitted for clarity). Values are estimates ± 1 SE (error bars). (*b*) Mean population size estimates per stream reach. Values are mean ± 95% bootstrapped CI (error bars).

both reference (1.16·m^{-2} (0.74–1.75, 95% CI)) and downstream reaches (1.08·m^{-2} (±0.36, 95% CI)) was significantly greater than larval density in the litter exclusion reach (0.26·m^{-2} (0.19–0.35, 95% CI)) (Fig. 2*b*).

Plots of mean biomass for the 1998–1999 cohort revealed linear growth patterns in each stream, indicating that growth was nearly continuous over the year (Fig. 3*a*). The slopes of the resulting significant growth rate regressions were compared among streams using analysis of covariance (ANCOVA) with time as the covariate. Regression slopes were significantly different ($p < 0.001$) among all stream reaches (reference > downstream > litter exclusion) (Fig. 3*a*).

Individual growth rates of all recaptured larvae were compared together over the study period because there was little evidence of seasonal influence on growth rate (Fig. 3*a*). Mean daily growth rates based on recaptured salamanders

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Fig. 2. (*a*) Monthly and (*b*) mean larval *Eurycea wilderae* density estimates in reference (solid circles and solid line), downstream of treatment (shaded triangles and dotted line), and litter exclusion (open circles and broken line) reaches from December 1997 to February 1999. Values are means ± 95% bootstrapped CI (error bars).

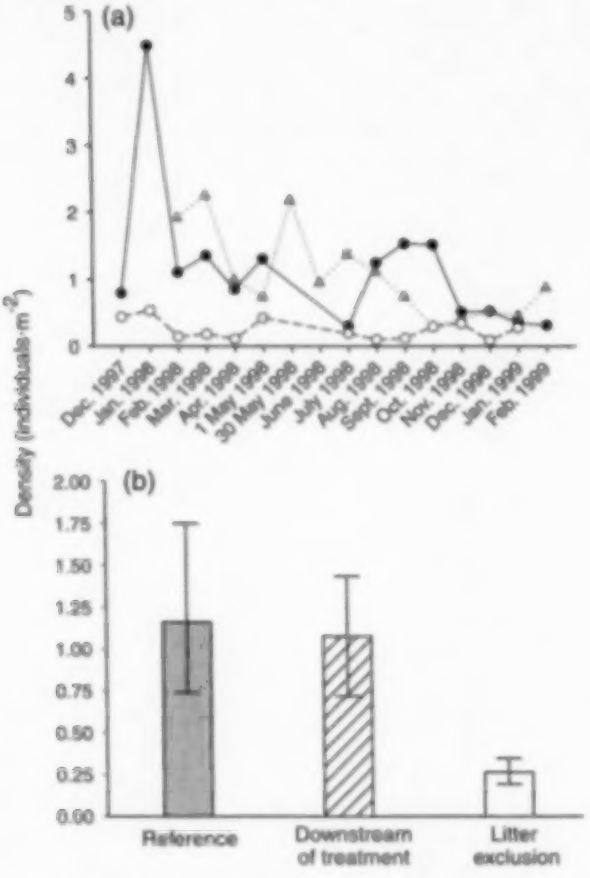
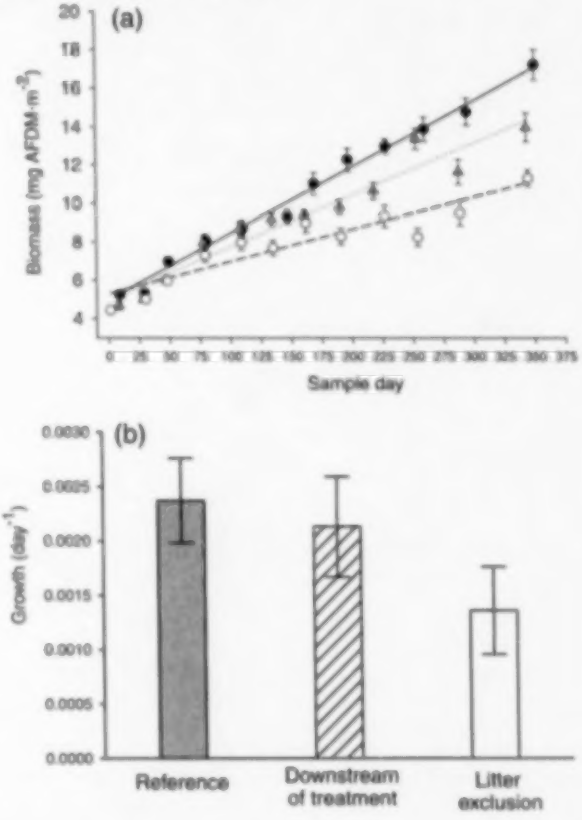


Fig. 3. (*a*) Relationship between time and *Eurycea wilderae* posthatching growth for the 1998–1999 cohort in reference (solid circles and solid line, $y = 0.034x + 4.991$), downstream of treatment (shaded triangles and dotted line, $y = 0.027x + 5.026$), and litter exclusion (open circles and broken line, $y = 0.017x + 5.288$) reaches. Regressions are based on mean biomass of all larvae collected on each sample date (day 0 = 1 May 1998 to day 351 = 19 February 1999). Values are means ± 1 SE (error bars). (*b*) Mean individual daily growth rates of all recaptured larvae in reference ($n = 122$), downstream of treatment ($n = 104$), and litter exclusion ($n = 99$) reaches. Values are means ± 95% bootstrapped CI (error bars).



were 0.0024·day^{-1} (±0.0004, 95% CI), 0.0021·day^{-1} (±0.0005, 95% CI), and 0.0014·day^{-1} (0.0004, 95% CI) for the reference, downstream of treatment, and litter exclusion reaches, respectively (Fig. 3*b*). By this method based on recaptured larvae, growth rate differences between the reference and litter exclusion reaches was significant, while the those of downstream reach was not significantly different from either.

Mean annual biomass in the reference and downstream reaches were 7.87 mg AFDM·m^{-2} (±0.19, 95% CI) and 6.62 mg AFDM·m^{-2} (±0.15, 95% CI), respectively. These estimates were greater than three times higher than that of the litter exclusion reach (1.96 mg AFDM·m^{-2} (±0.07, 95% CI)) (Fig. 4*a*). *Eurycea wilderae* production was 8.50 mg AFDM·m^{-2}·year^{-1} (±1.25, 95% CI), 7.35 mg AFDM·m^{-2}·year^{-1} (±1.44, 95% CI), and 1.27 mg AFDM·m^{-2}·year^{-1} (±0.33, 95% CI) in reference, downstream, and litter exclusion reaches, respectively (Fig. 4*b*). Production in the litter exclusion reach was therefore approximately one fifth that of reference and downstream reaches. Annual production to biomass (*P*/*B*) turnover ratios, which provide another measure of growth, were also significantly higher in the reference (1.1 (±0.13, 95% CI)) and downstream (1.1 (±0.22, 95% CI)) reaches than in the litter exclusion reach (0.65 (±0.17, 95% CI)).

Sixty *E. wilderae* larvae were collected for dietary analysis (reference, $n = 18$; downstream of treatment, $n = 23$; litter exclusion, $n = 19$), and a total of 34 prey taxa were identified in the stomachs. Diets of larvae in the reference reach showed little similarity to larval diets in either the downstream (68%) or the litter exclusion (53.1%) reach. MRPP analysis further showed that larval dietary composition in the reference reach was significantly different from both downstream ($p < 0.01$) and litter exclusion ($p < 0.001$) reaches. Diets of larvae in litter exclusion and downstream

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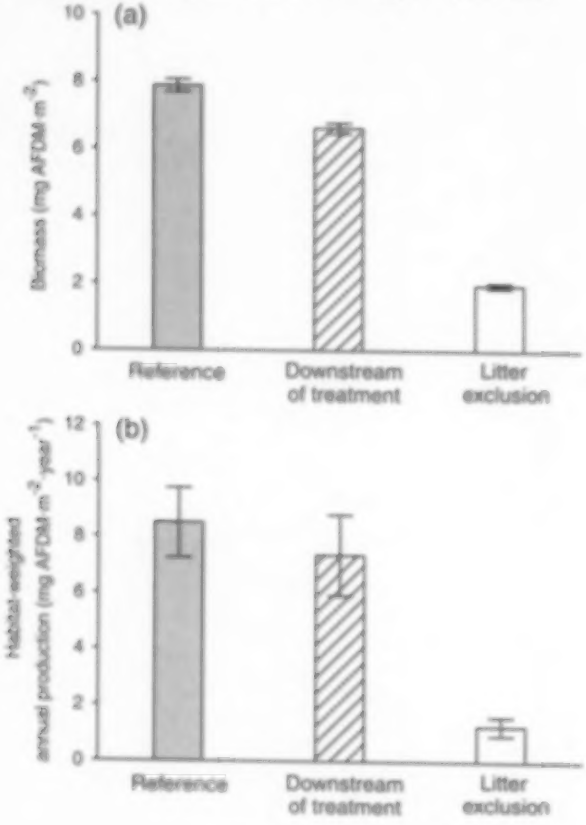
Fig. 4. (*a*) Mean annual biomass and (*b*) habitat-weighted annual production for the 1998–1999 larval *Eurycea wilderae* cohort in reference, downstream of treatment, and litter exclusion stream reaches. Values are means ± 95% bootstrapped CI (error bars).



reaches showed the highest similarity (78.6%) and were not significantly different ($p = 0.135$) based on MRPP analysis.

The 34 prey taxa were placed into 14 major categories for comparison among reaches (Table 3). Larvae in the reference reach had significantly more total prey items per gut (22.6 (16.78–29.28, 95% CI)) than larvae in the litter exclusion reach (11.95 (9.05–14.58, 95% CI)) (Fig. 5*a*). The reach downstream of treatment was intermediate in prey abundance (17.53 (14.43–21.17, 95% CI)) and was not significantly different from either of the other stream reaches. Copepods and non-Tanypodinae chironomids together accounted for an average of 77.1% of all prey items in *E. wilderae* diets in the three study reaches (Table 3). Larvae in the litter exclusion reach, however, ate fewer copepods and cladocerans and significantly more non-Tanypodinae chironomids, nematodes, and terrestrial insects than larvae in the reference reach (Table 3). Differences in total prey abundance between reaches were primarily due to the large number of copepods consumed by larvae in the reference reach.

There was no difference in total prey biomass per gut among reaches (reference, 0.28 mg (0.15–0.45, 95% CI); downstream, 0.55 mg (0.37–0.77, 95% CI); litter exclusion, 0.28 mg (0.18–0.39, 95% CI)) (Fig. 5*b*). Non-Tanypodinae

Table 3. Bootstrapped abundance (no. gut^{-1}), biomass (mg AFDM gut^{-1}), and percentages for prey items (no. of prey gut^{-1} and mass of prey gut^{-1}) in the diets of *Eurycea wilderae* larvae from reference (REF), downstream of treatment (DWN), and litter exclusion (LE) streams.

	Abundance			Biomass			Percent abundance			Percent biomass		
Prey category	REF	DWN	LE	REF	DWN	LE	REF	DWN	LE	REF	DWN	LE
Chironomidae*	5.1	7.39	6.25	0.063	0.106	0.108	24.77a	42.07ab	49.01b	30.80	30.26	52.86
Copepoda	14.37a	6.17b	2.8b	0.015A	0.006B	0.003B	59.49a	34.51b	21.30b	14.06A	3.55B	1.60B
Cladocera	0.54a	0.04ab	0b	0.001A	0B	0B	2.52a	0.31ab	0b	1.45A	0.10AB	0B
Nematoda	0a	0.22b	0.42b	0	0.001	0.009	0a	0.47ab	3.98b	0A	0.06A	2.85B
Terrestrials	0.06	0.13	0.47	0.001	0.007	0.005	0.09a	0.88a	5.80b	0.21A	0.87AB	3.83B
Other†	2.36	3.39	2	0.1974	0.442	0.155	13.53	21.91	19.59	53.2	64.78	38.47

Note: Significant differences are indicated by letters. Values within a prey category that are followed by the same lowercase letter (abundance columns) or uppercase letter (weight columns) are not significantly different based on overlap of bootstrapped 95% CIs.

*Non-Tanypodinae chironomids.

†Includes Ephemeroptera, Plecoptera, Trichoptera, Tanypodinae, Coleoptera, Ostracoda, Acarina, Oligochaeta, and other Diptera.

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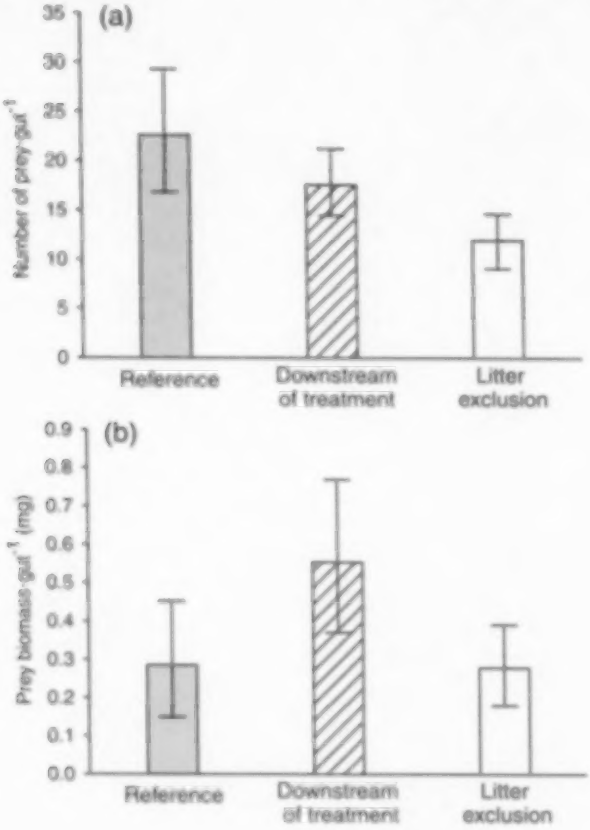
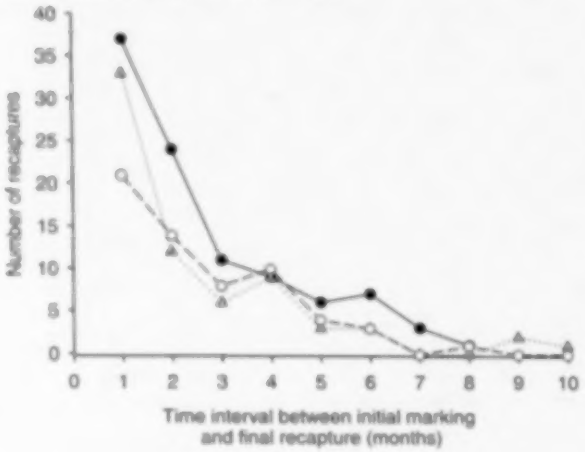
Fig. 5. (*a*) Total number of prey items and (*b*) total prey biomass per gut for larval *Eurycea wilderae* in reference (n = 18), downstream of treatment (n = 23), and litter exclusion (n = 19) stream reaches. Values are means ± 95% bootstrapped CI (error bars).



chironomids alone accounted for nearly a third of total prey biomass in reference and downstream reaches and more than half of prey biomass in the litter exclusion reach (Table 3). Larvae from the reference reach had greater biomass attributed to copepods and cladocerans, whereas litter exclusion larvae had significantly higher nematode and terrestrial insect biomass in guts. Mean prey biomass was slightly higher in the downstream reach owing to the presence of a few large stonefly larvae.

Mean movements for all recaptured larvae over the study period in reference, downstream, and litter exclusion reaches were only 2.38 m (1.24–3.75, 95% CI), 2.61 m (1.41–4.14, 95% CI), and 3.37 m (0.91–6.45, 95% CI), respectively, and there was no difference among reaches. This comparison is conservative, since the litter exclusion reach is the longest of the three reaches and the chance of capturing individuals that moved longer distances was therefore greater. Only 35.7% (n = 116) of recaptured larvae moved ≥1 m, and of those that moved, the majority (65.5%) moved in in the downstream direction. No larva moved >3 m upstream (mean = 1.25 m), while the greatest downstream movement was 93 m (mean = 11.04 m). In addition, only two recaptured larvae were found to have drifted from the litter exclusion reach into the reach downstream.

Fig. 6. Time interval between initial marking and final recapture for all *Eurycea wilderae* larvae recaptured in reference (solid circles and solid line, n = 122), downstream of treatment (shaded triangles and dotted line, n = 104), and litter exclusion (open circles and broken line, n = 99) reaches.



We used the time interval between initial marking and final recapture for all recaptured larvae as a surrogate for larval survivorship (Johnson and Wallace 2002) (Fig. 6). In all three reaches, recapture probability declined with time. Recapture interval data were transformed (log x + 1) and the resulting significant regression slopes (reference, $p < 0.001$; downstream of treatment, $p < 0.01$; litter exclusion, $p < 0.001$) were compared by ANCOVA with time as the covariate. There were no significant differences in survivorship of marked larvae among reaches (ANCOVA, $p = 0.23$).

Discussion

Eurycea wilderae density in the litter exclusion reach was <25% of reference and downstream reaches, despite having the largest wetted area. Reduced larval density in the treatment reach can result from greater mortality, emigration from the study reach, or reduced adult oviposition. Of the larvae that were captured and marked, there were no differences in survivorship among streams. Survivorship estimates resulted in typical type III survivorship curves (Deevey 1947) in all three streams. This survivorship pattern indicates high larval mortality and supports previously documented patterns for *E. wilderae* (Bruce 1988; Beachy 1997). Fewer initial captures in the litter exclusion stream combined with the lack of difference in survivorship of marked larvae suggest that lower larval density in the treatment stream is probably due to reduced oviposition or emigration of new hatchlings prior to initial marking.

Adult *Eurycea* feed primarily on terrestrial prey rather than on stream invertebrates (Burton 1976) and females typically oviposit on the underside of rocks rather than on in-stream woody debris or other organic matter (Baumann and Huels 1982; Bruce 1982). Therefore, treatment effects should have little influence on choice of oviposition sites.

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Long-term treatment effects (beginning in 1993), however, may have resulted in fewer adult females returning to the stream. If adults move little and return to the same streams to oviposit, reduced oviposition could contribute to lower densities in the treatment reach. Unfortunately, little is known of adult *E. wilderae* movements and home range size.

Numerous chamber experiments have investigated the effects of predation by larger salamander species on larval *Eurycea* (e.g., Gustafson 1993; Beachy 1994, 1997). Results from these enclosure studies are equivocal and must be interpreted with caution. While it is possible that predation by larger salamanders contributed to greater hatchling mortality in the litter exclusion stream where other prey items are scarce (Wallace et al. 1997, 1999), such strong predatory interactions seem unlikely given that even larger *E. wilderae* larvae (>12 mm) are vulnerable to predation, and if these interactions were common, they likely would have contributed to reduced survivorship of marked larvae as well.

Eurycea bislineata larvae have a strong tendency to drift downstream after hatching as a dispersal mechanism (Stoneburner 1978; Bruce 1986). Monthly drift data from the study streams also show greater spring drift of hatchling *E. wilderae* from the litter exclusion reach compared with the reference stream (T. Siler and J.B. Wallace, unpublished data). Reduced larval density in the litter exclusion stream therefore appears to be the result of greater hatchling drift in spring prior to initial capture and marking. Hatchlings are restricted to feeding mostly on copepods and other meiofauna owing to their small gape. Copepods declined dramatically following initiation of treatment (Wallace et al. 1999) and their scarcity, along with lack of detritus cover objects, could provide the additional drift stimulus for hatchlings.

Even though excess drift response may explain density differences among streams, we found no differences in movement of marked larvae in the streams. Optimal foraging theory (reviewed by Stephens and Krebs 1987) predicts that predators should have higher densities (numerical response) and spend more time (area-restricted search) in prey-rich patches than in prey-poor patches. Owing to the significant reduction of benthic invertebrate prey in the litter exclusion stream (Wallace et al. 1997, 1999), we initially hypothesized that salamander larvae would likely encounter fewer prey-rich patches and move more in search of prey. However, larvae in all three streams were largely sedentary. Mean larval movement of all recaptures in the study streams was only 2–3 m and nearly 65% of all recaptures moved <1 m. The same individuals were often recaptured in the same depositional areas several months in a row. As with larval *E. bislineata* (Bruce 1986), the majority of the movements were in the downstream direction. Upstream movements were short (< 3 m) and took place primarily in larger depositional areas where stream velocity was low. Only two of 104 larvae recaptured in the reach downstream of treatment were found to have drifted out of the litter exclusion stream. This indicates that larvae that did not drift soon after hatching tended to stay in the treatment stream, but at the cost of reduced fitness.

The pattern of larval growth after hatching indicated that growth occurred throughout the year in each of the streams with little influence of temperature. This linear growth pattern has been previously documented for *E. wilderae* larvae (Lugthart 1991; Beachy 1997; Johnson and Wallace 2002) and our daily growth rate of 0.0024 (±0.0004, 95% CI) for recaptured larvae in the reference stream closely agrees with a previous study that measured larval *E. wilderae* growth in this stream and in the litter exclusion stream prior to treatment using both in-stream chambers (reference, 0.003·day^{-1} (±0.001 SE); pretreatment litter exclusion, 0.003·day^{-1} (±0.001 SE)) and biomass regressions from field studies (reference, 0.004·day^{-1}; pretreatment litter exclusion, 0.003·day^{-1}) (Lugthart 1991). Even though results were similar, larval growth rates in chambers should be interpreted carefully because amphibian growth rates can be influenced by larval densities and prey availability (e.g., Petranka and Sih 1986; Walls 1998).

Two independent methods of growth measurement, both free from chamber effects, showed that litter exclusion resulted in significantly reduced *E. wilderae* growth. Hatchling biomass in May was very similar among streams, but by the following spring, larvae from the reference stream were an average of 52% larger than larvae from the treatment reach. Biomass regression slopes indicated that growth was significantly different among all three streams. Larval growth differences may have adverse consequences for *E. wilderae* population growth because larval growth can influence timing and size at metamorphosis and ultimately adult fecundity (Bruce 1982, 1988). Mortality also increases with duration of the larval stage (Bruce 1988). There were no obvious differences in timing or size at metamorphosis among streams, but sample sizes were too small for thorough analysis because collection of metamorphosing larvae was not an objective for this study.

Gut content analyses were performed after mark–recapture studies to explore the mechanism underlying reduced larval growth in the treatment stream. *Eurycea wilderae* fed predominantly on copepods and non-Tanypodinae chironomids. These two prey items accounted for >75% of all prey items in the guts in each stream. Similar findings were reported for *E. wilderae* in the study streams (Lugthart 1991) and for larval *E. bislineata* in an Indiana stream (Caldwell and Houtcooper 1973). Burton (1976) found that *E. bislineata* larvae at Hubbard Brook, New Hampshire, relied heavily on chironomid larvae but consumed few copepods.

Larval diets in the litter exclusion reach showed low similarity to diets of larvae from the reference stream primarily because larvae in the treatment reach ate fewer copepods and relied more on non-Tanypodinae chironomids and other prey groups. Benthic data confirm that copepods have declined by 95% of pretreatment values as a result of litter exclusion, whereas midges remain a relatively available food source (Wallace et al. 1999). Larvae in the litter exclusion stream also consumed significantly more unusual prey items such as nematodes and terrestrial insects. Unlike some studies that have that shown stream predators may rely heavily on prey from outside the system (Mason and MacDonald 1982; Nakano et al. 1999), in these streams, the terrestrial subsidy of prey was minor and apparently insufficient to support typical larval growth rates in the litter exclusion stream.

We initially hypothesized that food limitation was responsible for the reduction in *E. wilderae* growth because of the

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significant reduction in available prey items in the treatment stream (Wallace et al. 1997, 1999). Prey abundance in larval diets supported that hypothesis, but there were no differences in total prey biomass among reaches. The abundance of copepods in larval diets from the reference stream contributed heavily to the overall difference in prey number among streams. The lack of biomass difference resulted from low copepod biomass along with the fact that litter exclusion larvae consumed a greater number of larger non-Tanypodinae chironomids. Given the similarity of prey biomass among streams, the mechanism responsible for reduced growth in the treatment stream must be either a difference in prey quality or a difference in energetic demands.

Copepods may provide a nutritious food source for larvae in the reference stream. Lugthart (1991) found that growth of larval *E. wilderae* actually increased significantly following insecticide application in C54 at Coweeta. Gut content analysis revealed that abundance and biomass of copepods were considerably higher, although not significantly so, in the diets of larvae from the insecticide-treated stream compared with the reference stream and pretreatment diets (Lugthart 1991). Lipid reserves are the most efficient energy source for organisms, and copepods in pelagic systems can have up to 70% of their dry mass as lipids (Sargent and Falk-Petersen 1988). However, these planktivorous copepods derive their high lipid content from high-quality algal food resources (Sargent and Falk-Petersen 1988) that are virtually absent in shaded headwater streams at Coweeta. Further studies are needed to assess lipid content and assimilation efficiencies of salamander prey.

Reduced growth of larvae in the litter exclusion stream could also result from differences in energetic demand. Several studies have demonstrated a positive relationship between activity and larval amphibian growth (e.g., Werner 1991; Maurer and Sih 1996). Few studies, however, have evaluated effects of reduced prey availability on activity levels (Maurer and Sih 1996), and studies linking increased amphibian activity with energetic costs are lacking. The fact that salamanders in the treatment stream ate more nematodes and terrestrial insects strongly suggests that they have difficulty finding their preferred prey. If larvae exert more energy in searching for prey, then they may have less energy available for growth. Maurer and Sih (1996) found that larvae of the stream salamander *Ambystoma barbouri* actually reduced activity in response to food deprivation in a laboratory experiment, but the study was conducted for only 9 days and in complete absence of food. Even though we found no movement differences among streams, we measured movement only to the nearest metre and did not measure activity rates on a daily basis. In situ measurements of assimilation and production efficiencies would be required to verify differences in energetic demand among streams.

Eurycea wilderae production in Coweeta headwaters (~8 mg AFDM·m^{-2}·year^{-1}) is lower than that of many invertebrate groups (Wallace et al. 1999) because of their relatively low densities and slower growth rates. Yet, biomass and production were still significantly lower in the treatment stream as a result of reduced larval growth and density. The lower *P/B* turnover ratio in the litter exclusion stream is further evidence of reduced larval growth. Wallace et al. (1999) calculated annual production values for all larval salamander species from benthic samples over the period 1992–1997 in the reference and litter exclusion streams. Their reference and pretreatment salamander production values ranged from 93 to 336 mg AFDM·m^{-2}·year^{-1}, whereas production during treatment ranged only from 0 to 62 mg AFDM·m^{-2}·year^{-1}. Based on these estimates, *E. wilderae* comprises only a small portion of the total production of the salamander community in Coweeta streams and most production is attributed to *Desmognathus* spp. *Desmognathus* spp. larvae were also collected and marked during this study, but recapture rates were insufficient for population analyses. It is likely, however, that larval *Desmognathus* spp. are similarly affected by the litter exclusion treatment given their functional similarity to *E. wilderae*.

Many of the parameters measured for *E. wilderae* in the reach downstream of treatment (population density, growth, biomass, production, *P/B*, and prey abundance) had values that were intermediate between those for reference and litter exclusion reaches. These findings indicate that larvae in this downstream reach are still affected by the upstream litter exclusion treatment, possibly because prey availability remains low in this reach. Benthic samples were not collected in C56, but Baer et al. (2001) found that invertebrate colonization, secondary production, and fine particulate organic matter accumulation on artificial substrates were significantly reduced in this downstream reach after initiation of upstream treatment. Export of fine particulate organic matter from the upstream litter exclusion reach also declined by more than 81% over the treatment period (Wallace et al. 1999). The fact that *E. wilderae* larvae downstream of treatment showed adverse effects provides additional evidence for the importance of the detrital subsidy and upstream–downstream connections in lotic systems (e.g., Vannote et al. 1980; Wallace et al. 1982; Baer et al. 2001).

Hunter and Price (1992) referred to the effects of resources on higher trophic levels as a "cascade up" and several examples exist for living plant-based systems (e.g., Peterson et al. 1993; Harvey et al. 1998). The role of detritus in the classical food web framework has been largely overlooked (Polis and Strong 1996; Polis et al. 1997) and few experimental studies have demonstrated indirect effects of resources on predators in detritus-based ecosystems. Chen and Wise (1999) found that detritus enhancement led to higher densities of predaceous mites in a forest floor community, and Polis and Hurd (1995) found that marine-derived detritus facilitated terrestrial spiders on oceanic islands. In this study, long-term exclusion of the detrital resource produced strong indirect effects that resulted in reduced density, growth, biomass, and production as well as an altered diet of larval *E. wilderae*, a vertebrate predator. Removal of detritus altered invertebrate community structure such that it was incapable of supporting a typical *E. wilderae* population. More specifically, the dramatic decline in copepod density following litter exclusion treatment appears to be at least partly responsible for declines in *E. wilderae* growth and density. Our results provide the first comprehensive study of bottom-up effects of detritus manipulation on a predatory vertebrate species and demonstrate how loss of the detrital subsidy can affect species occupying higher trophic levels.

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Influence of moderate phosphate addition on nitrogen retention in an acidic boreal lake

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Abstract: Inorganic phosphorus (P) fertilizer was added to a small, acidic lake in southernmost Norway to test the potential of this technique to increase the retention of total inorganic nitrogen (TIN) and thereby mitigate the effects of elevated leaching of atmospherically derived N. The experiment was conducted over 4 years (1 pretreatment year followed by 3 years with weekly P additions during the growing season). To avoid any undesirable eutrophication effects, the total P concentration was increased from 4–5 μg P·L^{-1} to a moderate level of 10–12 μg P·L^{-1}. Compared with the pretreatment year, the P additions increased the average TIN retention in the experimental lake by nearly 70% (from 53 to 88 mmol·m^{-2}·year^{-1}) during three growing seasons. However, when considering that the reference lake experienced a 55% decrease in TIN retention during the same period, the increase might have been even larger. This underlines the role of P (both natural and anthropogenic) as an important regulator of N retention in upland lake districts that in large parts of southernmost Norway contribute significantly to the N loading on coastal marine areas.

Résumé : Nous avons ajouté un engrais à base de phosphore (P) inorganique à un petit lac acide du l'extrême sud de la Norvège afin de vérifier le potentiel de cette technique pour augmenter la rétention d'azote inorganique total (TIN) et ainsi mitiger les effets du lessivage accru de N d'origine atmosphérique. L'expérience a duré 4 ans, dont une année antérieure au traitement et trois années pendant lesquelles nous avons ajouté du P toutes les semaines durant la saison de croissance. Afin d'éviter les effets indésirables de l'eutrophisation, nous avons rehaussé la concentration totale de P de 4–5 μg P·L^{-1} à une concentration modérée de 10–12 μg P·L^{-1}. Par comparaison à l'année qui a précédé le traitement, les additions de P ont accru la rétention moyenne de TIN dans le lac expérimental par près de 70 % (de 53–88 mmol·m^{-2}·an^{-1}) durant les trois saisons de croissance. Cependant, si l'on tient compte du fait que le lac témoin a subi un déclin de 55 % de la rétention durant la même période, l'accroissement est sans doute plus élevé. Ces résultats soulignent le rôle de P (tant naturel que d'origine anthropique) comme important agent régulateur de la rétention de N dans les lacs des terres hautes qui, dans de grandes régions de l'extrême sud de la Norvège, contribuent de façon significative à l'enrichissement en N des zones côtières marines.

[Traduit par la Rédaction]

Introduction

Reductions by 50–60% in sulphur (S) deposition since 1980 have contributed significantly to a general trend towards recovery from acidification of European surface waters (Evans et al. 2001; Skjelkvåle at al. 2001*a*). In contrast, there have only been minor changes in nitrogen (N) deposition rates in the past two decades, and nitrate concentrations in surface water show no significant trends at most European long-term monitoring sites (Stoddard et al. 1999; Evans et al. 2001). This general pattern has led to increased focus on the fate of atmospheric N in terrestrial and aquatic ecosystems and the role of ammonium (NH_4^+) and nitrate (NO_3^-) in the acidification of soils and surface waters (Grennfelt and Hultberg 1986; Dillon and Molot 1990; Vitousek et al. 1997).

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Terrestrial ecosystems generally have large storage capacity for N (Nadelhoffer et al. 1999; Sogn et al. 1999). Despite this, large regions, including southern Norway, have experienced high NO_3^- concentrations in surface waters resulting from elevated levels of N deposition (Henriksen and Brakke 1988; Skjelkvåle et al. 2001*b*). In turn, this may contribute to surface water acidification (Reuss and Johnson 1986) and skewed stoichiometry of nutrient elements with possible eutrophication effects in rivers and sensitive coastal waters (Howarth et al. 1996; Hessen et al. 1997).

In catchments containing lakes, in-lake processes may contribute significantly to the combined terrestrial and aquatic retention of N compounds (Dillon and Molot 1990; Kaste and Dillon 2003). In acid-sensitive lakes, usually characterised by low total phosphorus (TP) concentrations, retention of N is mainly associated with phytoplankton assimilation (Baker and Brezonik 1988) or denitrification in the lake sediments (Rudd et al. 1986; Kelly et al. 1987). In both cases, the retention of N appears to be strongly related to water residence time. In lakes receiving elevated P inputs from anthropogenic sources, in-lake N retention seems to be more closely associated with lake water TP concentration than with residence time (Berge et al. 1997).

Experimental P addition to lakes has been carried out in a number of areas, especially in Canada. Most of the experiments have focused on nutrient dynamics and biological re-

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sponses (Schindler et al. 1973; Dillon et al. 1979) or on lake enrichment as a fishery management tool (Johannessen et al. 1984; Stockner and MacIsaac 1996; Johnston et al. 1999). Additionally, P has been applied to increase NO_3^- retention and neutralize acid lakes (Davison et al. 1995). In the latter study, it was demonstrated that by adding P to stimulate primary productivity and NO_3^- assimilation in a small and moderately acidified British upland lake, it was possible to reduce the acid (H^+) concentration to non-toxic levels for freshwater biota.

In 1998, the NITRAP project ("NITrate Retention in Acid lakes by Phosphate addition") was initiated to test the potential of P additions to increase N retention and reduce H^+ concentration in Norwegian acidic lakes. As a first step, various P doses were added to eight pelagic enclosures within the small and strongly acidified Lake Nedre Lundetjenn in southernmost Norway (Lyche-Solheim et al. 2001). Along a TP gradient of 4–19 $\mu g \cdot L^{-1}$ (0.13–0.61 $\mu mol \cdot L^{-1}$) and an exposure time of 3 weeks, they found that NO_3^- and H^+ concentrations in the enclosures decreased up to 13 $\mu mol \cdot L^{-1}$ and 6 $\mu mol \cdot L^{-1}$, respectively, corresponding to a complete retention of NO_3^- and a 50% decrease in acidity. To prevent the development of nuisance algae, the optimal TP concentration was found to be roughly 10–15 $\mu g \cdot L^{-1}$ (Lyche-Solheim et al. 2001).

On this basis, a 4-year whole-lake experiment was carried out in Lake Nedre Lundetjenn during 1998–2001, with additions of P fertilizer up to an environmentally acceptable lake TP concentration of ~12 $\mu g \cdot L^{-1}$ (~0.4 $\mu mol \cdot L^{-1}$) during three consecutive growing seasons. The main objective of this study was to evaluate the potential of a modest P enrichment as a possible management tool to increase in-lake N retention and thereby reduce N export to sensitive freshwaters and coastal marine recipients. Neutralising effects following the treatment were expected to be modest owing to high lake water H^+ concentration and buffering from organic anions. Here, we present chemical input and output data from the experimental lake and an upstream reference lake during the pretreatment year and the 3 years of treatment. The biotic responses to the enrichment are summarised in an accompanying paper (Donali et al. 2005).

Materials and methods

Site description

Lake Nedre Lundetjenn (58°22N, 8°27E), the experimental lake, and Lake Øvre Lundetjenn, the reference lake, are located near Grimstad, about 10 km from the southern coast of Norway. Water from the reference lake flows directly into the experimental lake through a narrow cement conduit (Fig. 1). The main physical and hydrological characteristics of the lakes are listed in Table 1. About 80% of the Øvre Lundetjenn catchment is drained by two main tributaries, Savalsbekken (1.00 km^2) and Myransbekken (0.61 km^2). Øvre Lundetjenn constitutes about 75% of the catchment draining into Nedre Lundetjenn. The study area (126–282 m above sea level) has a coastal climate with mean annual precipitation of about 1400 mm, a mean annual temperature of 7 °C, and usually a limited and irregular snow cover during winter. Precipitation amounts were distributed quite evenly between the growing season (1 May – 31 October) and the dormant season throughout the project period of 1998–2001 (Norwegian Meteorological Institute 2002). Growing-season air temperatures during same period were in the range of 12.2 to 13.6 °C, whereas the corresponding temperature range during the dormant seasons was 0.6–3.8 °C (Norwegian Meteorological Institute 2002).

About 60% of the catchment is covered by a mixture of coniferous and deciduous forests. The remaining area is mainly covered by small lakes, wetlands, and heathlands with small patches of bare rock. Bedrock is granite covered by thin and patchy podsolic and peaty soils. The area receives high levels of acid deposition. During 1998–2001, wet + dry S and N deposition at the closest monitoring station (Birkenes, about 11 km distant) was 7–9 and 13–19 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively (Norwegian Pollution Control Authority 2002). The ratios of oxidised N to reduced N in atmospheric deposition during 1998–2001 were 1.1 to 1.3. Apart from atmospheric deposition, there are no anthropogenic inputs of nutrients or organic substances to the lakes. The combination of slow-weathering bedrock and high levels of acid deposition has led to acidification of both study lakes and a subsequent loss of the native brown trout populations. During 2000 and 2001, in the reference lake Øvre Lundetjenn, mean (±standard deviation) H^+ concentration was 17.7 ± 7.3 $\mu mol \cdot L^{-1}$ (pH 4.75), mean inorganic aluminium (Al^{n+}) was 180 ± 70 $\mu g \cdot L^{-1}$, and mean acid-neutralising capacity (ANC) was −22.3 ± 26.9 $\mu equiv. \cdot L^{-1}$. The lakes are moderately affected by humic substances; the mean concentration of total organic carbon (TOC) at the outlet of Øvre Lundetjenn was 0.46 ± 0.14 $mmol \cdot L^{-1}$ (5.5 ± 1.7 mg $C \cdot L^{-1}$) during 2000–2001.

Experimental P additions to Lake Nedre Lundetjenn

The P-addition experiment was conducted over a 4-year period, which included 1 pretreatment year followed by 3 years of P addition (1999–2001). Each year, applications began in April–May and continued until October–November. The added P consisted of a solution of 1.45 $mol \cdot L^{-1}$ phosphoric acid (H_3PO_4) and 0.75 $mol \cdot L^{-1}$ sodium hydroxide (NaOH). The P additions were conducted to increase the mean epilimnetic P concentration from a background level of 0.10–0.15 $\mu mol \cdot L^{-1}$ (3–5 $\mu g \cdot L^{-1}$) to ~0.4 $\mu mol \cdot L^{-1}$ (~12 $\mu g \cdot L^{-1}$) during the growing season (May–October). The moderate level of P addition was chosen to avoid any eutrophication problems in the lake (Lyche-Solheim et al. 2001). The loading rates necessary for maintaining the epilimnion P concentrations near 0.4 $\mu mol \cdot L^{-1}$ were calculated on the basis of weekly TP analyses from the lake. Each week, the solution was applied evenly over the whole lake surface from a motorboat. During the 3 years of treatment, the P additions corresponded to 8.4, 4.6, and 5.3 $mmol \cdot m^{-2}$, respectively.

Hydrological budgets

Daily inputs and outputs of water from the lakes were estimated on the basis of streamwater discharge recorded at 6-h intervals at a gauging station at Savalsbekken, the main inlet of Øvre Lundetjenn. Weekly (summer) or biweekly (winter) records of water level in Nedre Lundetjenn show that the lake water level usually responds very rapidly (within 1 day) to changes in streamwater discharge. Hence, within-lake

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Fig. 1. Map showing the study lakes and their catchments in southern Norway.

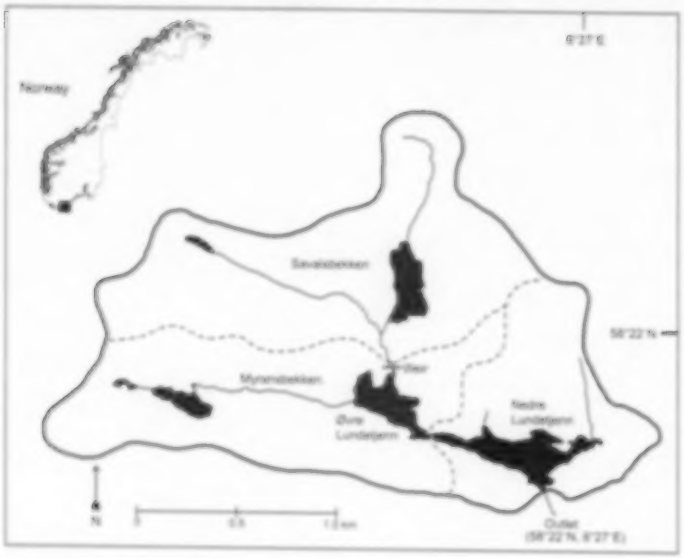

Table 1. Site characteristics of Lake Nedre Lundetjenn (the experimental lake) and Lake Øvre Lundetjenn (the reference lake).

	Nedre Lundetjenn	Øvre Lundetjenn
Lake surface (A_L), km^2	0.078	0.044
Catchment area (A_C), km^2	2.55	1.96
Average depth (Z_{mod}), m	8.5	7
Annual runoff (Q), m·year^{-1}	1.38	1.38
Residence time (T_w), years	0.19	0.11

Note: Runoff data refers to the period 1998–2001.

storage seems to have only minor effects on the hydrological dynamics at the outlet vs. the inlet streams in both Øvre and Nedre Lundetjenn. Accordingly, the daily discharge at all sampling points was estimated from the Savalsbekken gauging station, scaled in proportion to the respective catchment areas. As a test of the water balances, input–output budgets for chloride were calculated for 1 year (2000). These were within ±3% in both Øvre and Nedre Lundetjenn.

Chemical budgets

The inlets and outlets of Øvre and Nedre Lundetjenn (four stations) were sampled fortnightly during 1998–2001 (the inlet streams included from May 1998). The samples were analysed for several constituents, of which NO_3^-, NH_4^+, total N (TN), H^+, and TP are presented here. N species and TP were analysed by automated colorimetry: NO_3^- after reduction with cadmium amalgam, NH_4^+ after reaction with hypochlorite and phenol, TN after oxidation to NO_3^- with peroxidisulphate, and TP after oxidation with peroxidisulphate and reaction with molybdate and antimony. Detection limits for NO_3^-, NH_4^+, TN, and TP are 1, 5, 10, and 1 µg·L^{-1}, respectively.

Fluxes of N and P were calculated for each day using measured or estimated water discharges at all sampling points, and the corresponding chemical data were interpolated from the fortnightly samples. Likewise, flow-weighted means of a substance at a given sampling point were calculated by summing up the interpolated daily fluxes within a period (e.g., 1 year) and dividing by the accumulated flow for the same period (scaled from the Savalsbekken gauging station).

In-lake retention of N and P, expressed as mass (M) per unit of lake area, was calculated from the mass balances for the lakes:

$$M_{retained} = M_{in} - M_{out} - \delta S$$

where δS is the change in mass stored within the water column from the start to the end of the project period, M_{in} includes input from the terrestrial catchment plus atmospheric deposition on the lake surface, and M_{out} is loss at the lake outlet. δS was calculated from changes in the volume-weighted lake concentrations from spring overturn in 1998 to autumn overturn in 2000 (no vertical profile samples were available for 2001). During this period, concentration changes in both study lakes were <3 µmol·L^{-1} for NO_3^- and total organic nitrogen (TON), <1 µmol·L^{-1} for NH_4^+, and <0.04 µmol·L^{-1} for TP. The observed changes correspond to annual mean δS values of less than ±5 mmol·m^{-2}·year^{-1} for NO_3^- and TON, ±2 mmol·m^{-2}·year^{-1} for NH_4^+, and ±0.2 mmol·m^{-2}·year^{-1} for TP. These numbers are small compared with the annual retention estimates for the lakes, and δS was thus neglected in the calculation of chemical budgets. Owing to the short residence time of these lakes, the interannual variability might be higher than indicated above. Based on annual flow-weighted mean concentrations (see calculation above) at the outlet of the reference lake, the maximum interannual variation was 5.6 µmol·L^{-1} for NO_3^-, 1.8 µmol·L^{-1} for NH_4^+, and 0.05 µmol·L^{-1} for TP.

The terrestrial runoff comprises both sampled inflowing streams and ungauged diffuse runoff to the lakes. Because the catchments are relatively homogenous in land-cover characteristics, the fluxes measured at the terrestrial catch-

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ment Myransbekken were extrapolated to the ungauged portion of the catchment. Atmospheric deposition of N and P was estimated from the monitoring station Birkenes (Oredalen and Aas 2000; Norwegian Pollution Control Authority 2002). At Birkenes, dry deposition of N constituted 9–14% of the wet deposition during 1998–2001. As dry deposition commonly is higher in forested vs. open areas, N deposition on the lake surfaces was assumed to be wet only, determined by the chemical composition and amount of precipitation in daily samples (Tørseth and Semb 1998).

Uncertainties in the lake budgets

Uncertainties in the input–output budgets of the lakes are mainly associated with possible errors in (*i*) water budgets, (*ii*) estimates of atmospheric deposition, (*iii*) chemical budgets attributable to stream sampling frequency, and (*iv*) estimation of fluxes from ungauged areas. These possible sources of error in the lake budgets are discussed in detail elsewhere (Kaste and Dillon 2003).

Results

Pretreatment year: 1998

The experimental lake Nedre Lundetjenn and the upstream reference lake Øvre Lundetjenn share about 75% of the total catchment area and thus exhibit very similar chemical characteristics (Fig. 2). Both lakes have low TP concentrations, with averages of 0.14–0.18 $\mu mol \cdot L^{-1}$ (~4–6 $\mu g \cdot L^{-1}$) during 1998 (Table 2). The study area is heavily affected by acidification as a result of high levels of N and S deposition. Average H^+ concentrations during 1998 were 14.3 and 17.5 $\mu mol \cdot L^{-1}$ (pH 4.84 and 4.76) in Nedre and Øvre Lundetjenn, respectively. Relatively low retention capacity for N deposited from the atmosphere (total inorganic N retention around 70%; Kaste and Dillon 2003) has led to elevated N concentrations in the lakes and their tributaries. During the pretreatment year, average NO_3^- concentrations in Nedre and Øvre Lundetjenn were 18.9 and 21.2 $\mu mol \cdot L^{-1}$, respectively. The ratios of NO_3^- to $NO_3^- +$ non-marine SO_4^{2-} in the lakes (Henriksen and Brakke 1988) indicate that about 30% of the anthropogenic acidification during 1998 was due to NO_3^- leaching from the terrestrial catchment. Hence, the fate of inorganic N (TIN) within the catchment and in the lakes has important implications for acidification status in the lakes.

Both lakes showed typical seasonal patterns in the concentrations of N species and H^+ (Fig. 2). The NO_3^-, NH_4^+, TON, and H^+ levels generally were of comparable magnitude in the two lakes over the course of the pretreatment year. The most significant difference between the lakes was found for H^+ during the summer months, when concentrations in Nedre Lundetjenn occasionally were up to 6 $\mu mol \cdot L^{-1}$ lower than in Øvre Lundetjenn. There was also a tendency towards higher NO_3^- concentrations in the reference lake during the dormant season. In general, NO_3^- was the most important N species, with typical values around 10 $\mu mol \cdot L^{-1}$ during summer and 25–35 $\mu mol \cdot L^{-1}$ during winter. TON showed the opposite seasonal pattern and usually exceeded NO_3^- during the growing season, with concentrations reaching 20 $\mu mol \cdot L^{-1}$. NH_4^+ concentrations typically were in the range of <1 to 5 $\mu mol \cdot L^{-1}$ during 1998.

Treatment period: 1999–2001

Following the additions of P to Nedre Lundetjenn, average TP concentrations during the growing season increased from 0.15 $\mu mol \cdot L^{-1}$ in 1998 to 0.32–0.40 $\mu mol \cdot L^{-1}$ during 1999–2001 (Fig. 3). Because of short residence time, however, the effect of P addition on the lake water TP level was a transient phenomenon: after termination of the P additions each autumn, the TP concentrations declined rapidly to the pretreatment level. Hence, the dormant-season TP level remained low and fairly constant throughout the whole study period.

As hypothesised, the P additions resulted in a significant decrease in summer NO_3^- concentrations in Nedre Lundetjenn. Compared with Øvre Lundetjenn, which maintained a minimum NO_3^- level of ~10 $\mu mol \cdot L^{-1}$ during the growing season, NO_3^- concentrations in Nedre Lundetjenn decreased to levels of 3.8, 4.4, and 1.9 $\mu mol \cdot L^{-1}$ during 1999 to 2001, respectively (Fig. 2). On average, there was a slight decrease (~7%) in the growing-season NO_3^- concentrations in Nedre Lundetjenn from 1998 to 1999–2001, whereas concentrations in the reference lake increased by about 6% during the same period (Table 2). During the dormant season, NO_3^- concentrations in Nedre and Øvre Lundetjenn were of comparable magnitude throughout the study period. The elevated NO_3^- concentration observed during winter in 2000–2001 probably was an effect of an extremely wet autumn (October–November) followed by a long period with dry, cold weather and extensive soil frost development. High soil solution and surface water NO_3^- concentrations associated with severe soil frost events are also reported elsewhere (Mitchell et al. 1996; Fitzhugh et al. 2001; Kaste and Skjelkvåle 2002).

NH_4^+ and TON concentrations seemed to be largely unaffected by the P addition, whereas the H^+ response is difficult to evaluate owing to high intersite and temporal variability. H^+ concentrations generally were lower in Nedre vs. Øvre Lundetjenn during the growing season, but the average δH^+ during the growing seasons of 1999–2001 was around the same level (~3 $\mu mol \cdot L^{-1}$) as in the year before treatment. The concentrations of inorganic aluminium (Al^{in}) in Nedre Lundetjenn largely followed the same pattern as H^+ during 1998–2001, and the growing-season Al^{in} concentrations generally were up to 50 $\mu g \cdot L^{-1}$ lower than in the reference lake (data not shown). With seasonal Al^{in} concentrations in the range of 50 to 350 $\mu g \cdot L^{-1}$, however, the water quality in Nedre Lundetjenn was still toxic for fish during most of the year (Lien et al. 1996).

In-lake retention of N and P

According to an empirical N retention model for acid-sensitive lakes (Kelly et al. 1987), the retention coefficient of NO_3^- is inversely related to the ratio of lake mean depth to theoretical water residence time (Z_{mid}/T_W). With Z_{mid}/T_W ratios of 45 $m \cdot year^{-1}$ in Nedre Lundetjenn and 61 $m \cdot year^{-1}$ in Øvre Lundetjenn, the former lake is expected to be slightly more efficient in retaining NO_3^-. During the pretreatment year, the estimated NO_3^- retention (relative to the input) was 17% in Nedre Lundetjenn and 10% in Øvre Lundetjenn (Table 3).

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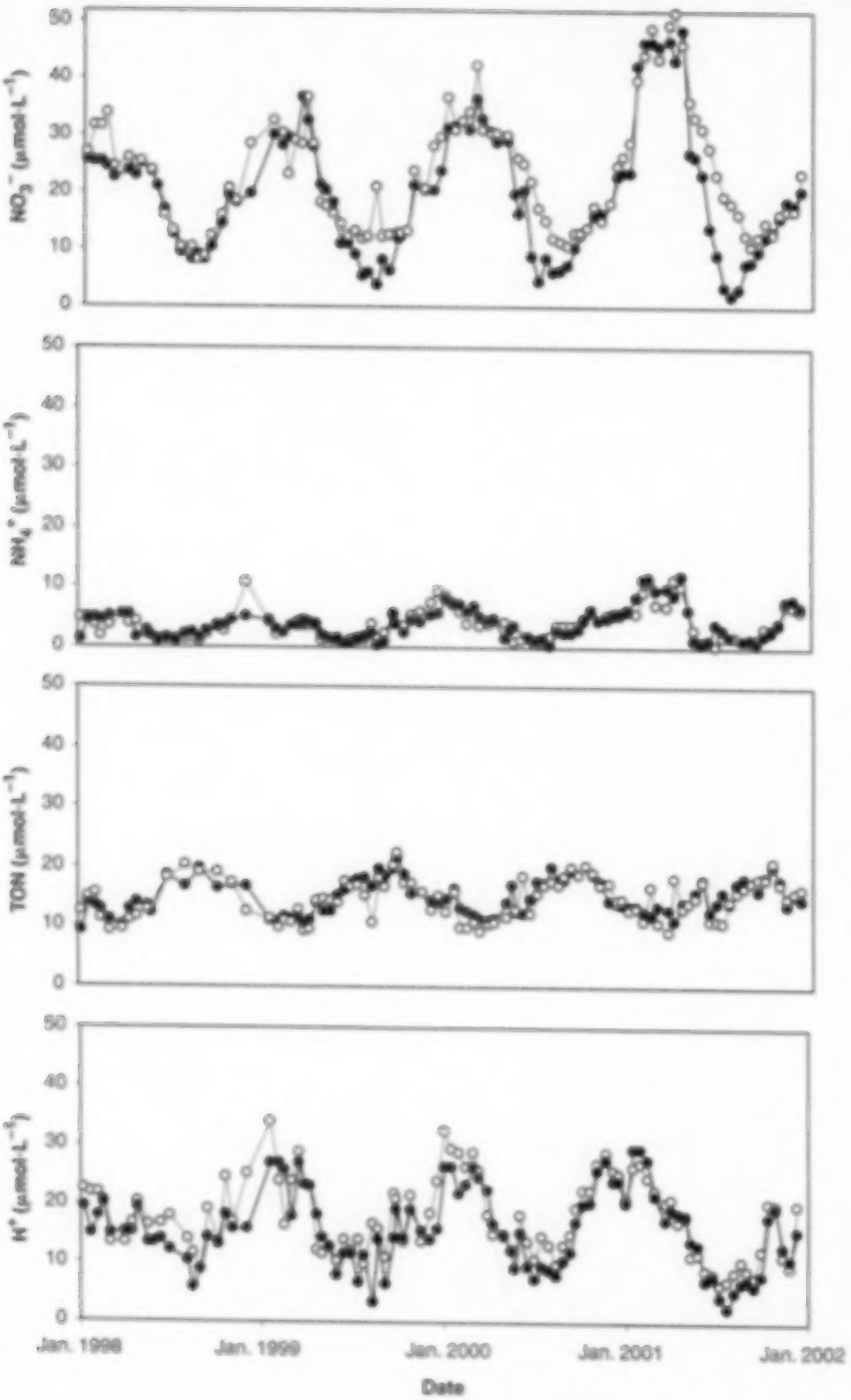
Fig. 2. Time series showing NO_3^-, NH_4^+, total organic nitrogen (TON), and H^+ concentrations in Nedre (—●—) and Øvre (—○—) Lundetjenn.



The NH_4^+ and TP balances in the study lakes were rather different in that Øvre Lundetjenn appeared to be a net source for both elements. One known source is the accumulation and subsequent loss of NH_4^+ and phosphate (PO_4^{3-}) from a deep bottom water layer (16–19 m) that usually becomes anoxic during the summer and winter stagnation (Dillon and

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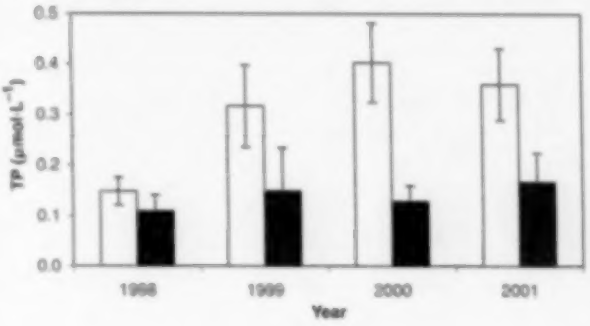
Table 2. Flow-weighted mean concentrations (μmol·L^{-1}) of chemical compounds at the outlets of Nedre Lundetjenn and Øvre Lundetjenn (reference) during the pretreatment (1998) and treatment (1999–2001) years. Growing season is defined as 1 May – 31 October.

	Nedre Lundetjenn		Øvre Lundetjenn	
	1998	1999–2001	1998	1999–2001
(*a*) Growing season				
NO_3^-	13.9	12.9	14.8	15.7
NH_4^+	2.6	2.9	2.3	3.3
TP	0.14	0.29	0.18	0.20
H$^+$	11.4	14.1	14.7	16.8
(*b*) Annual basis				
NO_3^-	18.9	22.2	21.2	24.2
NH_4^+	3.3	4.9	4.1	5.1
TP	0.13	0.21	0.14	0.17
H$^+$	14.3	19.0	17.5	21.1

Fig. 3. Arithmetic mean total phosphorus (TP) concentrations in Nedre Lundetjenn as a response to experimental P additions carried out during the growing season (May–October) in 1999–2001. Growing-season samples (open bars) are from the epilimnion (0–4 m), dormant-season samples (solid bars) are from the outlet.



Molot 1996; Donali et al. 2005). Another possible source for the excess NH_4^+ and TP could be the 0.35-km^2 ungauged catchment surrounding the Øvre Lundetjenn. As a rough test on this, a small brook draining approximately 20% of this area was included in the sampling programme on four occasions (one per season) in 2001. Based on these samples, however, there was no significant difference in NH_4^+ concentration levels (TP not measured) between the small brook and the two main tributaries of Øvre Lundetjenn. This indicates that NH_4^+ fluxes estimated from the gauged inlet streams were also fairly representative of the ungauged parts of the catchment. This suggests that internal release of NH_4^+ and PO_4^{3-} from anoxic lake sediments in Øvre Lundetjenn was the most likely source responsible for the difference in NH_4^+ and TP balance between the two lakes.

Following P additions to Nedre Lundetjenn during 1999–2001, the retention of TP during the growing season increased from 0.6 mmol·m^{-2} in 1998 to 1.0–5.9 mmol·m^{-2} after treatment (Table 3). The retention efficiency, relative to the total inputs, varied from 20% in 1998 to 16%–63% in 1999–2001. The apparent increase in percentage P retention with increasing P enrichment might be due to higher bioavailability of the added phosphate fertilizer than to the background P loading, which to a large extent was made up of less available dissolved or particulate organic P (Donali et al. 2005). Compared with the year before treatment, the additions of P to Nedre Lundetjenn during 1999–2001 resulted in a significant increase (*t* test performed on monthly values, $p < 0.1$) in the in-lake retention of NO_3^- and NH_4^+ during the growing season. In terms of mass retained per unit of surface area, the increase from 1998 to 1999–2001 was 57%–71% for NO_3^- and 45%–109% for NH_4^+. Compared with the reference lake, the retention of NO_3^- in Nedre Lundetjenn nearly tripled during the growing seasons of 1999–2001. On an annual basis, however, the P additions (carried out in May–October of each year) had only minor effects on the NO_3^- and NH_4^+ budgets. Even though TON generally was lost from the lake systems, there was a net retention of TN in Nedre Lundetjenn, both within the growing season and on an annual basis during 1999–2001 (131–182 mmol·m^{-2}·year^{-1}).

Discussion

The underlying hypothesis for this experiment is that moderate P additions to Nedre Lundetjenn during the growing season will increase the assimilation of TIN and thus the overall in-lake retention of NO_3^- and NH_4^+. If utilised mainly by algae, approximately 16 moles of N will be assimilated for each mole of P taken up (Redfield 1958). Considering that uptakes of NO_3^- and NH_4^+ have opposite effects on the acid–base balance of terrestrial and aquatic ecosystems (Reuss and Johnson 1986), the effects from P additions on lake water acidity thus depend on the relative amounts of NO_3^- and NH_4^+ assimilated and the buffering capacity of the water.

Observed vs. potential chemical changes

During 1999–2001, the P additions to Nedre Lundetjenn corresponded to 8.4, 4.6, and 5.3 mmol·m^{-2}, respectively (mean ±1 standard deviation (SD); 6.0 ± 2.0 mmol·m^{-2}). This was nearly three times the background P loading during the growing seasons of 1998–2001 (2.3 ± 1.1 mmol·m^{-2}). If fully used by the primary producers and given the Redfield ratio, the background P loading would imply a N uptake of 37 ± 17 mmol·m^{-2}. This is close to the mass-balance NO_3^- retention estimate for 1998 (42 mmol·m^{-2}), which also includes denitrification. In reality, a large fraction (70%–80%) of the background TP loading was organic P with low bioavailability (e.g., associated with humic material), and the actual N retention that could be accounted for by the background P loading probably was relatively small. To illustrate this, the mean chlorophyll carbon concentrations in the experimental lake before and during the treatment were 1.0 ± 0.1 and 4.4 ± 1.2 μg·L^{-1}, respectively (Donali et al. 2005). Also, aquatic vegetation growth was sparse, with the exception of a small transition area between the reference and treatment lakes. Observations from the experimental lake Nedre Lundetjenn indicated an increased periphyton growth after treatment (Marit Mjelde, Norwegian Institute for Water

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Table 3. In-lake retention of NO_3^-, NH_4^+, total organic nitrogen (TON), and total phosphorus (TP) in Nedre and Øvre Lundetjenn in 1998–2001 (*a*) during the growing season (May–October) and (*b*) on an annual basis.

	NO_3^-	NH_4^+	TON	TP
(*a*) Growing season				
Nedre Lundetjenn				
1998	42 (15)	11 (20)	Nd (Nd)	0.6 (20)
1999	67 (26)	22(36)	–17 (–8)	5.9 (63)
2000	72 (23)	16 (20)	–23 (–8)	1.0 (16)
2001	66 (27)	23 (40)	–10 (–4)	3.8 (45)
1999–2001 mean	68 (26)	20 (31)	–17 (–7)	3.6 (44)
Øvre Lundetjenn				
1998	48 (12)	1 (2)	Nd (Nd)	–1.1 (–33)
1999	28 (8)	0 (–1)	–38 (–13)	–0.3 (–10)
2000	45 (11)	–17 (–22)	–94 (–27)	–1.7 (–52)
2001	–1 (0)	7 (11)	8 (2)	1.1 (23)
1999–2001 mean	24 (7)	–3 (–5)	–41 (–12)	–0.3 (–8)
(*b*) Annual basis				
Nedre Lundetjenn				
1998	150 (17)	50 (29)	Nd (Nd)	0.5 (9)
1999	178 (17)	43 (22)	–39 (–7)	6.7 (46)
2000	166 (14)	26 (9)	–60 (–8)	2.2 (17)
2001	141 (13)	6 (2)	–16 (–3)	3.7 (28)
1999–2001 mean	162 (14)	25 (10)	–38 (–6)	4.2 (31)
Øvre Lundetjenn				
1998	119 (10)	–31 (–18)	Nd (Nd)	–2.8 (–59)
1999	98 (7)	–35 (–16)	–76 (–11)	–0.8 (–11)
2000	199 (12)	–42 (–14)	–186 (–19)	–3.3 (–41)
2001	146 (9)	–27 (–9)	–46 (–6)	–0.1 (–1)
1999–2001 mean	148 (9)	–35 (–13)	–103 (–13)	–1.4 (–16)

Note: Missing data from the two inlet streams in Øvre Lundetjenn during January–April 1998 are substituted by the 1999–2001 mean for the same streams and months, respectively. The retention values are presented as mass per unit lake surface (mmol·m^{-2}) with percentage of inputs in brackets. Nd, no data.

Research, P.O. Box 173, Kjelsås, N-0411 Oslo, Norway (marit.mjelde@niva.no), unpublished data). These are qualitative or semiquantitative observations, however, and cannot be used directly to assess the importance of macrophyte and periphyton vs. phytoplankton growth.

In theory, a complete utilisation of the experimentally added phosphate fertilizer by primary producers would have increased the TIN retention by 74–134 mmol·m^{-2}. According to the water budgets for the individual treatment years, this corresponds to a reduction of TIN concentrations at the outlet of Nedre Lundetjenn by 4.2–9.5 µmol·L^{-1} during the growing season and 1.4–3.3 µmol·L^{-1} on an annual basis. If NO_3^- and NH_4^+ are assimilated in a proportion of 4:1 during the growing season, then the potential H^+ reduction following the treatment is estimated to be 33–59 mmol·m^{-2} (H^+ contribution from the fertilizer itself included). This corresponds to a reduction of H^+ concentrations at the outlet by 1.9–4.2 µmol·L^{-1} during the growing season and 0.6–1.5 µmol·L^{-1} on an annual basis. When considering the high pretreatment H^+ concentrations in the lake, the P additions were expected to have a relatively small influence on pH values in the water.

As could be expected from the short residence time in Nedre Lundetjenn, the added P was not fully retained within the lake. Using the TP retention estimate for 1998 as a rough baseline for the whole experimental period, we estimate that about 10%–60% of the P added was retained during the consecutive growing seasons of 1999–2001. In addition, some of the added P might have been assimilated and subsequently lost as organic P via the lake outlet.

Although there was a significant increase in TIN retention after P addition to Nedre Lundetjenn, the increment was somewhat less than the theoretical potential (see above). Using the TIN retention estimate for 1998 as a baseline, the growing-season TIN retention increased by 35–36 mmol·m^{-2} (or roughly 25%–45% of the theoretical potential) following the treatment. The difference between the theoretical and observed TIN retention can be due partly to severe P limitation and satiation of the NO_3^- needs in the algal cells before the treatment combined with light-limited primary production after treatment, preventing maximal NO_3^- uptake (Donali et al. 2005). Considering that denitrification is a relatively slow process compared with assimilation, it is also possible that denitrification rates were suppressed by NO_3^- uptake during the treatment period (Kelly et al. 1990).

There was an inverse relationship between flushing rate and NO_3^- retention (Fig. 4). During dry periods with low flushing rate (<0.5 epilimnion water replacements per month), up to 80%–90% of the monthly NO_3^- input was retained. In periods with high flushing rates (>2 epilimnion re-

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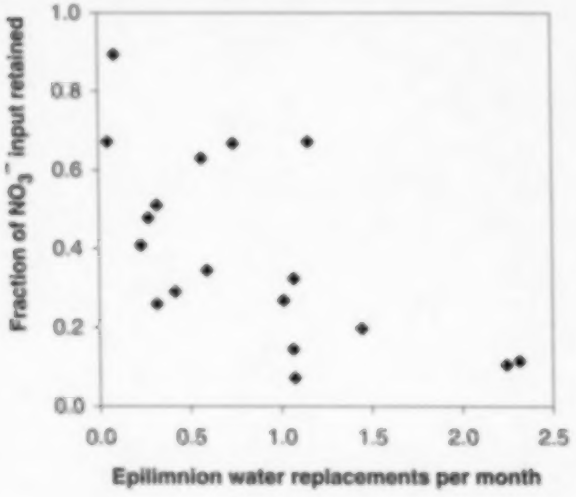
Fig. 4. Monthly NO_3^- retention fraction vs. estimated epilimnion water replacement time during the growing season (May–October) in Nedre Lundetjenn, 1999–2001.



placements per month), the monthly NO_3^- retention decreased below 10%, also during the growing season. Owing to the generally short residence time of the lake system, the effects from P addition were significant only during the growing season, whereas the annual NO_3^- and NH_4^+ budgets were largely unaffected. With the exception of short periods with high NO_3^- retention and low water inputs from the terrestrial catchment, there was no clear H^+ response during the 3 years of treatment.

Compared with a 3-week mesocosm experiment performed at Nedre Lundetjenn in 1998 (Lyche-Solheim et al. 2001), the present whole-lake experiment gave roughly similar results. In fertilized enclosures with TP concentrations of 0.4 µmol·L^{-1} and 0.5 µmol·L^{-1} (close to the levels in Nedre Lundetjenn after treatment), NO_3^- concentrations decreased from an initial level of 13 µmol·L^{-1} to 9.3 µmol·L^{-1} and 6.4 µmol·L^{-1}, respectively. This is slightly less than the maximum reduction obtained during the summer months in Nedre Lundetjenn after P addition. The reductions of H^+ concentrations obtained in the same enclosures were modest, 1–3 µmol·L^{-1}. By adding higher doses of P (final concentration 0.65–0.90 µmol·L^{-1}) during two consecutive summer seasons, Davison et al. (1995) managed to reduce the H^+ concentration by about 5 µmol·L^{-1} (from pH 5.2 to 5.9) in a small, acidic British upland lake. The lake water NO_3^- concentrations were concurrently reduced from a relatively low pretreatment level of 2.0–3.5 µmol·L^{-1} to values below the detection limits.

Fate of retained inorganic N

The major TIN removal processes in lakes are denitrification in anoxic sediment microlayers, assimilation by primary producers, and sedimentation (Ahlgren et al. 1994). Whereas the NO_3^- fraction that undergoes denitrification is lost to the atmosphere mainly as dinitrogen (N_2) and nitrous oxide (N_2O), the assimilated TIN is transformed to TON and stored in standing crops, sedimented, or lost via the lake outlet. The sedimented fraction might be buried for a long time or mineralised to NH_4^+.

During the growing seasons of 1999 and 2000, denitrification rates in littoral and profundal sediments were measured in both Øvre Lundetjenn and Nedre Lundetjenn (Haande 2001). Denitrification rates in littoral sediments were examined by in situ incubations in 39-L Plexiglas chambers enclosing the sediment surface. To avoid significant contribution of primary producers to NO_3^- retention, the chambers were located on sediment surfaces with no visible macrovegetation. Six 2-week incubation periods (three chambers in each lake) were carried out during the growing seasons of 1999 and 2000. Within the same study, profundal sediment cores (two from each lake, sampled eight times during the growing seasons of 1999 and 2000) were carried back to the laboratory and incubated for 3 weeks. In both cases, the denitrification rates were obtained from measured NO_3^- concentrations in the overlying water at the beginning and end of each incubation period. The results showed that growing-season denitrification rates were of the same magnitude in both lakes during 1999 and 2000, with mean values in the range of 0.14 to 0.20 mmol·m^{-2}·day^{-1} NO_3^- (Haande 2001). There were also relatively small differences between denitrification rates measured in littoral vs. profundal sediments. If considering these values as fairly representative for both lakes, the growing-season denitrification losses might have been in the range of 25 to 35 mmol·m^{-2} during the study period. In that case, denitrification might have contributed 35%–50% and 55%–100% to the in-lake NO_3^- retention in Nedre Lundetjenn and Øvre Lundetjenn, respectively, during the growing seasons of 1999–2000.

Fertilisation of Nedre Lundetjenn during the growing seasons of 1999–2001 resulted in a substantial increase in phytoplankton biomass and primary production (Donali et al. 2005), leading to a higher potential for algal NO_3^- uptake. Despite this large response of the phytoplankton community, no undesirable eutrophication effects or species occurred. Mean N sedimentation rates in Nedre Lundetjenn during the growing seasons of 1999–2001 were ~110 mmol·m^{-2} (Donali et al. 2005), i.e., slightly higher than the total estimated TIN retention in Nedre Lundetjenn during 1999–2001 (88 mmol·m^{-2}). This indicates that some of the sedimented TON was mineralised (recycled) during the production period. The estimated TON loss via the lake outlet corresponded to 10–23 mmol·m^{-2} during the growing seasons in 1999–2001. Some fraction of this might also contain N associated with algal production.

Can moderate P addition to lakes mitigate negative effects of elevated N leaching?

Leaching of atmospheric N from terrestrial ecosystems has caused acidification and nutrient imbalance (e.g., high N/P ratios) in many lakes and rivers in southern Norway over the past 2–3 decades (Henriksen and Brakke 1988; Faafeng and Hessen 1993). The experiments performed in Nedre Lundetjenn during 1999–2001 demonstrated that moderate P addition (to lake water concentration ~0.4 µmol·L^{-1} TP) increased the in-lake TIN retention by nearly 70% during the growing season and re-established a more natural N:P ratio at the outlet. The lack of a clear pH response after P addition underlines the limitation of this

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technique to de-acidify strongly acidic (pH <5.0) and humic lakes. However, in more moderately acidified clear-water lakes (e.g., pH 5.2–5.5), the potential H^+ reduction following the treatment (1.9–4.2 $\mu mol \cdot L^{-1}$) might be sufficient to alter the lake water pH.

The P-addition experiment performed in Nedre Lundetjenn demonstrates that significant increases in lake N retention can be achieved without causing undesirable eutrophication effects. This underlines the important role of P (both natural and anthropogenic) as a resource and a regulator of N retention in upland lake districts that in large parts of southernmost Norway contribute significantly to the N loading on coastal marine areas.

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Pelagic response of a humic lake to three years of phosphorus addition

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Abstract: Three years of whole-lake phosphorus (P) fertilization, conducted in a 12-ha boreal forest lake, revealed significant changes in epilimnion nutrients, biomasses, and primary production. As a time average for all three treatment years, primary production increased 257% relative to the reference basin value of 16.5 $mg \cdot m^{-3} \cdot day^{-1}$, whereas the carbon masses of both nanoalgae (>2 µm) and zooplankton increased roughly 130% from their initial values of 15 $mg \cdot m^{-3}$ and 17 $mg \cdot m^{-3}$, respectively. Calculated from a difference, the absolute increase in the sum of ciliates and picoplankton (heterotrophic bacteria and picoalgae) was more than six times as large as for algae larger than 2 µm, indicating that most added P ended in this compartment. Moreover, fertilization did not change the species inventory among nanoalgae and zooplankton, although the biomass composition changed somewhat. Only the former dominant species, the chrysophytes *Dinobryon crenulatum*, *D. sociale* v. *americanum*, *Mallomonas allorgei*, and *Ochromonas* sp. and the calanoid copepod *Eudiaptomus gracilis* increased substantially in biomass owing to the added P. Surprisingly, we observed a substantial delay in the food web response to fertilization, where most variables increased monotonously in size from year to year during the fertilization period. The underlying mechanisms for these delayed increases remains to be explained.

Résumé : Un enrichissement en phosphore de la masse d'eau complète pendant 3 années dans un lac de 12 ha de la forêt boréale a provoqué des changements significatifs des nutriments de l'hypolimnion, des biomasses et de la production primaire. En moyenne sur les trois années de l'expérience, la production primaire a augmenté de 257 % par rapport à la valeur de référence du bassin de 16,5 $mg \cdot m^{-3} \cdot jour^{-1}$, alors que les masses du carbone des nanoalgues (>2 µm) et du zooplancton ont toutes deux augmenté d'environ 130 % à partir de valeurs initiales respectives de 15 $mg \cdot m^{-3}$ et de 17 $mg \cdot m^{-3}$. Calculée d'après la différence, l'accroissement absolu de la somme des ciliés et du picoplancton (bactéries hétérotrophes et picoalgues) a été de plus de six fois plus important que celui des algues de taille supérieure à 2 µm, ce qui indique qu'une grande partie du P ajouté a abouti dans ce compartiment. De plus, la fertilisation n'a pas changé la liste des espèces des nanoalgues et du zooplancton, bien que la composition de la biomasse ait été un peu altérée. Seules les espèces dominantes d'origine, les chrysophytes *Dinobryon crenulatum*, *D. sociale* v. *americanum*, *Mallomonas allorgei* et *Ochromonas* sp. et le copépode calanoïde *Eudiaptomus gracilis* ont subi un accroissement substantiel de biomasse en réaction à l'addition de P. De façon inattendue, nous avons observé un important délai dans la réaction du réseau alimentaire; la plupart des variables ont en effet crû de façon graduelle d'année en année durant la période de fertilisation. Les mécanismes qui sous-tendent ces accroissements retardés restent à découvrir.

[Traduit par la Rédaction]

Introduction

Phosphorus (P) plays a central role in lake ecosystems, and its effect on food web structure and function, as well as on lake metabolism, has been studied extensively (Vollenweider 1968). Whereas a main problem for numerous lakes is overload of P, leading to eutrophication characterized by nuisance algal blooms and anoxia (Smith 1998), deliberate addition of P has recently been introduced as an alternative to liming of acidic lakes and also as a method for reducing the N export to sensitive coastal areas (Davison et al. 1995; Lyche-Solheim et al. 2001). The rationale behind the method is that algal production in most freshwater bodies is P-limited (Schindler 1980). Addition of P will therefore lead to increased algal photosynthesis and growth, thereby increasing uptake of nitrate, which has been shown to generate base and increase water pH (Schindler et al. 1985; Davison et al. 1995). However, such additions will potentially have numerous side effects that must be carefully mapped and understood under a wide range of conditions before P fertilization can be used on a large scale for practical or managemental purposes.

Most studies dealing with the effects of P additions have been performed in clear-water lakes (Schindler 1975, 1980). However, high levels of dissolved organic carbon are likely to modify the ecosystem response. These compounds, mostly humic substances of terrestrial origin (Mcknight and Aiken

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1998), often dominate the organic carbon in oligotrophic rivers and lakes (Hessen and Tranvik 1998).

The presence of humic compounds can reduce primary production in the water column physically by reducing the light penetration (Carpenter et al. 1998) and chemically by making trace elements and P less available for the phytoplankton (Jones et al. 1998). The biotic effects of allochthonous carbon on lake productivity are mediated by both energetic and trophic mechanisms. Allochthonous carbon can be an important energy source for zooplankton, directly by zooplankton feeding on particulate allochthonous detritus (Poulet 1983) and indirectly by zooplankton feeding on bacteria that have utilized dissolved allochthonous detritus (Tranvik 1998). Moreover, allochthonous carbon may also have a strong influence on the competition between bacteria and algae for inorganic P (Jansson 1998). When allochthonous carbon is abundant, the bacteria become more P-limited and will thus compete effectively, because of their large surface area to volume ratio, with the phytoplankton for P (Vadstein 2000). Conversely, for phytoplankton species in humic lakes, bacterivory may be an important strategy for acquiring P or other nutrients in short supply (Nygård and Tobiesen 1993), as well as for fighting their competitors for these substances. Moreover, a large pool of recalcitrant dissolved C may also render these lakes more stable with dampened consumer–resource oscillations (Wetzel 1995). Conversely, it is not as straightforward to predict the potential effect of added P, and its fate within the food web, in a humic lake as in a clear-water lake.

The aim of this article is to present and discuss the responses of the pelagic biota in an acidic boreal forest lake to 3 years of P fertilization. A more detailed analysis of the chemical responses is given elsewhere (Kaste and Lyche-Solheim 2005). Initially, the experiment was conducted to test if moderate P addition is an environmentally acceptable means of increasing the retention of N in acidic boreal forest lakes, thereby expanding the findings by Davison et al. (1995) based on data from a clear-water lake.

Materials and methods

Site description

The P-addition experiment was conducted in Lake Lundetjenn, a 12-ha oligotrophic and acidic lake in a forested catchment area in southern Norway (58°22′N, 8°26′E). The lake is divided into two subbasins, hereafter referred to as the reference and treatment basins. The morphometric and hydrological data for the two lake basins are given in Table 1. Two main inlet streams flow into the reference basin, and the outlet from the reference basin is the main input to the treatment basin. The two basins are separated by a narrow sound 40 to 50 m long, and we could not measure any transfer of added P from the lower treatment basin to the upper reference basin. The background concentration of total P in Lake Lundetjenn was roughly 5 $mg \cdot m^{-3}$, and the water was humic with a DOC concentration of 4–5 $g \cdot m^{-3}$ (Table 2).

P addition

For each treatment period (the summers of 1999, 2000, and 2001), P was added weekly from 1 May to 31 October by running boat transects across the treatment basin while slowly emptying a large tank of diluted phosphoric acid into the surface waters. The epilimnion P concentration was kept at 10–12 $mg \cdot m^{-3}$ in the treatment basin during these years, roughly twice as high as the pretreatment P concentration in both basins (Table 2). The weekly dose of added P was estimated from the water inflow rate, recorded at an automatic recording station in one of the main inlet streams to the reference basin, and the measured total P concentration in the mixed layer of the treatment basin (taken roughly 2 days after each weekly P addition).

Table 1. Morphometrical and hydrological data for the two basins of Lake Lundetjenn.

Parameter	Reference	Treatment
Catchment size (km^2)	1.96	2.55
Altitude (m)	126	126
Lake surface area (km^2)	0.044	0.078
Maximal depth (m)	19	25
Mean depth (m)	7.0	8.5
Volume ($\times 10^6$ m^3)	0.308	0.659
Runoff ($\times 10^6$ $m^3 \cdot year^{-1}$)	1.978	2.573
Specific runoff ($L \cdot s^{-1} \cdot km^{-2}$)	32	32
Residence time (days)	58	95

Note: The runoff and specific runoff data refer to the 30-year mean for 1961–1994.

Water sampling and analysis

During the whole project period (1998–2001), water samples were taken every 2 weeks from May to October from a station situated above the deepest point in each lake basin. At each visit, the mixed layer (0–4 m) was sampled twice with a flexible-tube sampler (diameter 7 cm, volume 15 L), and the samples were pooled. Subsamples were filtered on precombusted and HCl-washed glass-fibre filters (Whatman GF/F) and dried at 60 °C overnight, and the collected filtrates were stored for later analysis of dissolved organic carbon (DOC) (Abdullah and Eek 1996). The filters were either packed in tin capsules and analysed for particulate carbon and nitrogen (POC and PON, respectively) on an elemental analyser (Carbo-Erba 1106 CHN, Milano, Italy) or analysed for particulate phosphorous (POP) as described below. Subsamples were also taken for analysing dissolved inorganic carbon (DIC) (Abdullah and Eek 1995), inorganic nitrogen (NO_3^- and NH_4^+), and total phosphorous (TP). N species and TP were analysed by automated calorimetry: NO_3^- after reduction with cadmium amalgam, NH_4^+ after reduction with hypochlorite and phenol, and TP after oxidation with peroxidisulphate and reaction with molybdate (Parsons et al. 1992). Subsamples for phytoplankton and zooplankton quantification were fixed with Lugol's solution immediately after sampling, stored in the dark, and counted and measured according to conventional techniques to obtain data on biomass and species composition (Utermöhl 1958; Rott 1981; Bottrell et al. 1976). Only algae larger than 2 μm were enumerated and will hereafter be referred to as the nanoalgae, although several of these species are too big to fit the 2- to 20-μm size category. The upper part of hypolimnion (4–8 m) was also sampled for zooplankton biomass and species composition.

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Table 2. Values (seasonal mean ± standard error) for variables measured in the reference and treatment basins during the treatment period (1999–2001).

	1998		1999–2001		
Variable	Reference	Treatment	Reference	Treatment	Unit
TP	5.88±0.00	4.00±0.00	6.0±0.9	10.7±1.2	$mg \cdot m^{-3}$
NH_4^+	16.86±1.16	24.0±2.7	27.3±5.3	20.3±5.2	$mg \cdot m^{-3}$
NO_3^-	174.3±7.5	154.8±1.6	251±23	148±21	$mg \cdot m^{-3}$
DIC	Nd	Nd	1.1±0.3	0.5±0.2	$mg \cdot m^{-3}$
DOC	Nd	Nd	5.2±0.3	4.4±0.3	$g \cdot m^{-3}$
POC	885±55	896±31	948±86	1126±131	$mg \cdot m^{-3}$
PON	60.9±0.4	55.3±4.3	82.7±11.8	119±19	$mg \cdot m^{-3}$
POP	4.35±0.07	3.49±0.00	5.8±1.1	9.7±1.4	$mg \cdot m^{-3}$
POC:PON	14.5±0.9	16.4±0.7	14.9±10.3	10.2±2.7	$mg \cdot mg^{-1}$
POC:POP	203±10	258±7	174±20	122±16	$mg \cdot mg^{-1}$
pH	4.81±0.00	5.27±0.17	4.91±0.09	5.07±0.12	—
A_n	8.95±1.19	10.9±1.0	14.6±2.0	33.8±12.9	$mg \cdot m^{-3}$
Chl	1.48±0.05	1.0±0.1	1.61±0.18	4.36±1.22	$mg \cdot m^{-3}$
Chl:A_n	0.135±0.012	0.095±0.002	0.127±0.040	0.188±0.008	$mg \cdot m^{-3}$
Z	20.5±4.3	14.7±2.8	17.3±5.0	40.3±15.2	$mg \cdot m^{-3}$
PP	Nd	Nd	16.5±12.1	58.9±39.1	$mg \cdot m^{-3} \cdot day^{-1}$
SE_C	Nd	Nd	18.9±5.4	26.9±11.3	$mg \cdot m^{-3} \cdot day^{-1}$
SE_N	Nd	Nd	1.3±0.7	1.8±0.7	$mg \cdot m^{-3} \cdot day^{-1}$
SE_P	Nd	Nd	0.09±0.05	0.13±0.02	$mg \cdot m^{-3} \cdot day^{-1}$

Note: All samples are taken from the epilimnion (0–4 m) during the growing season (1 May – 31 Oct). Abbreviations: TP, total phosphorus; POC, PON, POP, particulate organic carbon, nitrogen, and phosphorous, respectively; DIC, DOC, dissolved inorganic and organic carbon, respectively; NH_4^+, ammonia N; NO_3, nitrate N; A_n, nanoalgal carbon; Z, zooplankton carbon; Chl, chlorophyll carbon; PP, primary carbon production rate; SE_C, SE_N, SE_P, sedimentation rate of particulate carbon, nitrogen, and phosphorous, respectively; Nd, no data available.

Rate measurements

Primary production was measured by the standard ^{14}C method (Parsons et al. 1992). Light and dark bottles (9 and 2, respectively, 60 mL) were filled from the pooled water sample, tracer was added, and the bottles were then suspended between the surface and a depth of 2 m in the lake for 24 h starting after sunset. After incubation, 10-mL subsamples from each bottle were acidified (1 drop 37% HCl) overnight to remove nonassimilated $^{14}CO_2$, scintillation cocktail (Ultima Gold XR, Perkin-Elmer, Boston, Mass., 12 mL) was added, and the subsamples were stored to let the chemiluminescence die out (>8 h) before the radioactivity (dpm) was counted on a scintillation counter (Tri-Carb 4000, Packard, Ill.). Quenching was corrected for with the internal standard method. Average primary production for epilimnion ($mg \cdot m^{-3} \cdot day^{-1}$) was estimated by trapezoid integration of the production values found at each depth.

Sedimentation was measured with sediment traps suspended at a depth of 10 m at the main station in each of the two lake basins and harvested every 2 weeks. The traps were made of Plexiglas cylinders (50 cm high with an inside diameter of 7 cm) giving an aspect ratio of >7. The trap material was filtered and analysed for C, N, and P according to the analytical techniques described above.

Statistics

Analysis of variance (ANOVA) was used to analyse the log-transformed time-series data. Type-III sum of squares (Venables and Ripley 1999), which calculates the significance of a model factor when all other factors are included in the model, was applied. This reduces the risk of confounding the effects of different variables and makes the test more conservative compared with type-I sum of squares, where the significance of a model factor depends on the order by which the factors are entered into the model. For 1998, the year before fertilization, the model factors were lake basin B (levels: reference, treatment) and month of the year M (levels: 1, 2, ..., 12). This model was used to judge if there was any significant differences between the basins before fertilization. For 1999–2001, the model factors were treatment T (levels: nonfertilized, fertilized), year Y (levels: 1999, 2000, 2001), and month of the year M (levels: 1, 2, ..., 12), as well as the two-way interactions $T \times Y$ and $T \times M$. The choice of this particular model was based on a preliminary analysis in which level of model complexity gave the overall best fit for all response variables. The rational for involving year and month as explanatory factors is to correct the data for the influence of these two sources of variability to more clearly judge the significance of fertilization effects.

The ANOVA for the 1998 data (not shown) revealed no basin differences in any of the response variables. Thus, any significant effect of fertilization found by ANOVA from the 1999–2001 data could be argued to be due to fertilization alone and not partly due to any inherent basin differences. ANOVA assumes that the observations are independent and normally distributed with equal variances (Sokal and Rohlf 1995). Normal probability plots of the residuals and plots of the residuals versus the fitted values showed that the assumptions of normality and equal variances were reasonably

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Table 3. The effect of P fertilization, as revealed by analysis of variance (ANOVA), for a select choice of log-transformed variables.

Variable	T	Y	M	$T \times Y$	$T \times M$	R^2
TP	**0.000**	**0.000**	0.031	0.251	**0.005**	0.71
NH_4^+	**0.001**	0.006	**0.000**	0.044	0.350	0.61
NO_3^-	**0.000**	0.240	**0.000**	**0.001**	**0.001**	0.82
POC	0.052	**0.000**	0.006	0.093	0.520	0.56
PON	**0.001**	**0.000**	0.055	0.294	0.760	0.54
POP	**0.000**	**0.000**	0.013	0.790	0.050	0.72
A_n	**0.000**	**0.000**	**0.000**	0.751	0.645	0.71
Z	**0.000**	**0.003**	**0.000**	0.025	0.909	0.62
PP	**0.000**	0.615	**0.000**	0.263	0.488	0.81
SE_C	0.026	0.125	**0.002**	0.286	0.151	0.54

Note: For each variable, the p values of the main effects treatment T, year Y, and month M, as well as for the two-factor interactions $T \times Y$ and $T \times M$, are given. The Bonferoni method (Sokal and Rohlf 1995) was used to ensure that the overall experimentwise error rate did not exceed $\alpha = 0.05$. This was done by using a significance level for each individual test equal to $\alpha' = \alpha/n$, where $n = 10$ is the number of variables tested. Significant terms at the $p < 0.05$ level are shown in bold. For each response variable, the explained sum of squares, R^2, for the ANOVA model is also given. Abbreviations: TP, total phosphorus; POC, PON, POP, particulate organic carbon, nitrogen, and phosphorous, respectively; DIC, DOC, dissolved inorganic and organic carbon, respectively; NH_4^+, ammonia N; NO_3^-, nitrate N; A_n, nanoalgal carbon; Z, zooplankton carbon; Chl, chlorophyll carbon; PP, primary carbon production rate; SE_C, sedimentation rate of particulate carbon.

fulfilled. Because the data are time series, we could have expected serial-correlated residuals, invalidating the assumption of independent model errors. However, for most response variables, we did not find any significant serial correlation of the residuals, and for a few variables, we found serial correlation at lags larger than 1 month, which were judged as very unlikely and coincidental. The Bonferoni method (Sokal and Rohlf 1995) was used to insure that the overall experimentwise error rate did not exceed $\alpha = 0.05$. This was done by using a significance level for each individual test equal to $\alpha' = \alpha/n$, where $n = 10$ is the number of variables tested.

Results

Chemistry and seston

Because the treatment basin received about 75% of the water input from the reference basin, the two basins exhibited much of the same chemical characteristics before P addition (Table 2). Both basins were heavily affected by acidification from slow-weathering bedrock and relatively high levels of N and S deposition (Kaste and Lyche-Solheim, unpublished data). During 1998, the two basins had pH values of 4.81 ± 0.00 and 5.27 ± 0.17, respectively, averaged over the whole season and over the depth interval of 0–4 m (Table 2).

As a result of the P additions, TP increased significantly in the treatment basin relative to the reference basin (Tables 2 and 3) and more than doubled from 4.0 ± 0.0 $mg \cdot m^{-3}$ in 1998 to 10.7 ± 1.2 $mg \cdot m^{-3}$ as an average value for 1999–2001. The reference basin also experienced an increase in TP after the onset of fertilization, although a modest one. There was also a significant effect of fertilization on the concentration of inorganic nitrogen (Table 3). Although the reference basin experienced a substantial increase in mean growing season total inorganic nitrogen (TIN) concentrations during 1998–2001, there was almost no change in TIN in the treatment basin during the same period. Thus, it seems clear that the TIN concentrations measured in the treatment basin during 1999–2001 were depressed as a response to the P fertilization.

The added P resulted in small relative increases in particulate organic carbon, nitrogen, and phosphorus (POC, PON and POP, respectively) (Table 2), which were significant only for PON and POP (Table 3). As 3-year averages for the period 1999–2001, the PON was 82.7 ± 11.8 $mg \cdot m^{-3}$ and 119 ± 19 $mg \cdot m^{-3}$ for the reference and treatment basins, respectively, whereas the corresponding figures for POP were 5.8 ± 1.1 $mg \cdot m^{-3}$ and 9.7 ± 1.4 $mg \cdot m^{-3}$ for the reference and treatment basins, respectively. These changes resulted in notable basin differences in particulate organic matter (POM) C:N:P stoichiometry (Table 2), being approximately 174:12:1 and 122:12:1 in the reference and treatment basins, respectively. Furthermore, during the same 3 years, the stoichiometry of the POM change, defined as ΔPOC:ΔPON:ΔPOP, was 46:9:1, where Δ denotes the difference between the treatment and reference basins.

Phytoplankton

The nanoalgal community was characterized by few dominant species and limited seasonal variability. The chrysophytes *Dinobryon crenulatum*, *D. sociale* v. *americanum*, *Mallomonas allorgei*, and *Ochromonas* sp. were the most abundant species in both basins during the whole investigation period, whereas chlorophytes, cryptophytes, dinophytes, and diatoms played only a minor role (Fig. 1). The number of observed species was roughly 50–60 for both basins and all years, and more than 80% of the species were found in both basins. For both basins and for all years covering the period 1998–2001, the seasonal changes in the nanoalgal biomass (A_n) showed a pronounced increase after ice melt at the beginning of May, peaking in mid-June, followed by a decrease during July, and a steady low biomass from the onset of August and thereafter (Fig. 2). The classical second

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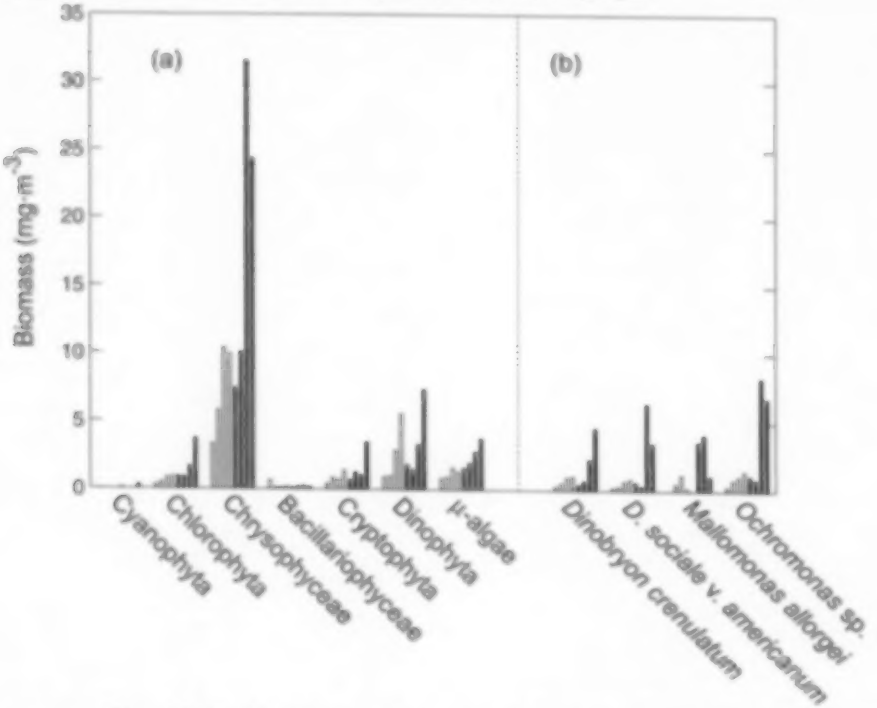
Fig. 1. The taxonomic composition of the phytoplankton community: (*a*) composition with respect to main algal groups, (*b*) composition with respect to the four most-dominating algal species. The open and solid bars represent biomass values (carbon) in the reference basin and treatment basin, respectively. For each, the four bars represent the years 1998, 1999, 2000, and 2001, respectively, when read from left to right. Unspecified algae within size range of 2–4 μm are abbreviated as μ-algae.



minor bloom in the autumn (Wetzel 2001) did not take place in any of the basins for any of the sampling years.

Fertilization made the algal biomass increase significantly (Tables 2 and 3). In 1998, before P addition, both basins had similar algal carbon masses, approximately 10 mg·m^{-3} (Fig. 3). During fertilization, the treatment basin had an algal biomass more than two times larger than that in the reference basin, as an average for 1999–2001 (Table 2). Furthermore, the difference between the two basins increased steadily from 1999 to 2001. However, this increased response over the years did not result in any significant interaction between treatment and year in the ANOVA model (Table 3).

During all 3 years, with fertilization the difference between the basins with respect to nanoalgal biomass was highest during the midsummer algal peak period and almost vanished in the late summer and autumn periods (Fig. 2). The increased algal biomass was mainly due to an increase in the dominant chrysophyte species, but other species also increased compared with the reference basin (Fig. 1). Moreover, the weight ratio of measured chlorophyll over estimated nanoalgal carbon (Chl/A_n; mg·mg^{-1}) also seemed to increase but was not included in the ANOVA (Table 2; Fig. 3).

Zooplankton

In both basins and for all four investigated years, the metazoan community was dominated by the calanoid copepod *Eudiaptomus gracilis*, constituting 80%–90% and 65%–90% of the seasonal average zooplankton biomass in the reference and treatment basins, respectively (Fig. 4). Cladocerans and rotifers made up <15% and <8% of the seasonal mean zooplankton biomass in the reference and treatment basins, respectively. Among the cladocerans, *Diaphanosoma brachyurum* and *Holopedium gibberum* were most common, whereas *Bosmina longispina* was less abundant in both basins. *Daphnia* spp. and cyclopoid copepods were absent in both basins. The micro filter feeder *Kellicottia longispina* was the dominant species among the rotifers. The phantom midge *Chaoborus* spp. was often found in the zooplankton samples and showed moderate variations from year to year in both basins. Two species were identified, *C. flavicans* and *C. obscuripes*, of which the latter occurred most frequently in both basins. Largest biomass for all zooplankton groups was usually observed in the 0- to 4-m depth interval, roughly twice the biomass in the 4- to 8-m interval. In 1998, before fertilization, the zooplankton communities in the reference and treatment basins were very similar, with seasonal mean carbon biomass of 20.5 ± 4.3 and 14.7 ± 2.8 mg·m^{-3}·day^{-1}, respectively.

Zooplankton biomass increased significantly as a result of fertilization (Tables 2 and 3). As an average for 1999–2001, the zooplankton carbon biomasses in the treatment and reference basins were 40.3 ± 15.2 and 17.3 ± 5.0 mg·m^{-3}, respectively. Moreover, the treatment basin response increased over the years, being 0%, 78%, and 295% larger than the reference basin values for 1999, 2000, and 2001, respectively (Fig. 3). The dominant zooplankton, the calanoid *E. gracilis*, accounted for more than 85% of this increase,

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Fig. 2. The seasonal time series for those variables exhibiting most seasonal variability in the reference (triangles) and treatment (circles) basins. Abbreviations and units: A_n, nanoalgal carbon ($mg \cdot m^{-3}$); Z, zooplankton carbon ($mg \cdot m^{-3}$); Chl, chlorophyll carbon ($mg \cdot m^{-3}$); NO_3^-, nitrate N ($mg \cdot m^{-3}$); PP, primary carbon production rate ($mg \cdot m^{-3} \cdot day^{-1}$).

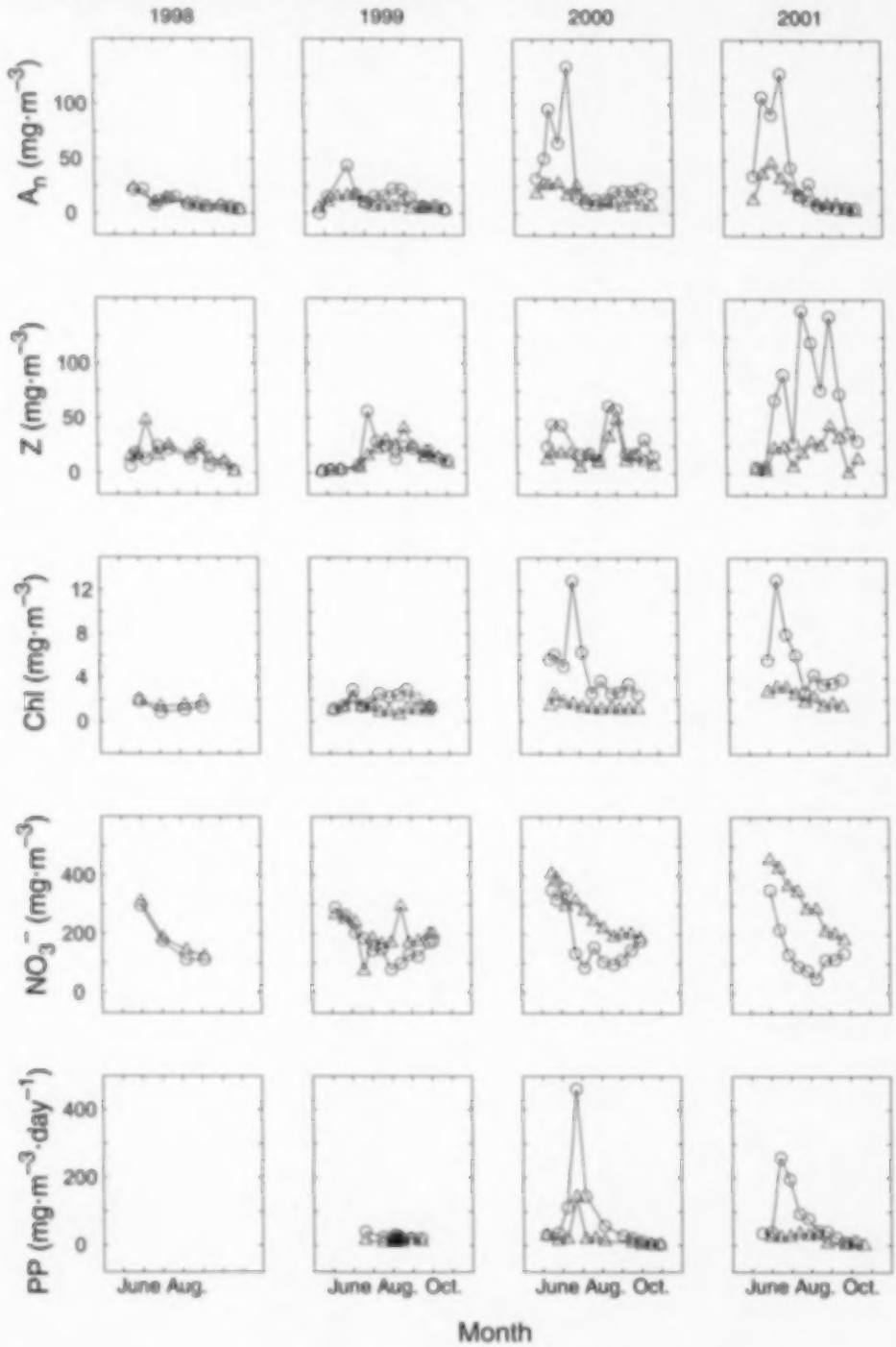

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Fig. 3. Measured variables, expressed as seasonal means ± standard error for the reference (triangles) and treatment (circles) basins. The values for the two basins are offset by 0.1 year to increase the readability of the figure. Abbreviations and units: TP, total phosphorus ($mg \cdot m^{-3}$); DIN, dissolved inorganic nitrogen, NO_3^- and NH_4^+ ($mg \cdot m^{-3}$); A_n, nanoalgal carbon ($mg \cdot m^{-3}$); Chl, chlorophyll carbon ($mg \cdot m^{-3}$); PP, primary carbon production rate ($mg \cdot m^{-3} \cdot day^{-1}$); A_n/TP, nanoalgal carbon per total phosphorus unit (unitless); Chl/A_n, chlorophyll carbon per nanoalgal carbon unit (unitless); Z, zooplankton carbon ($mg \cdot m^{-3}$).

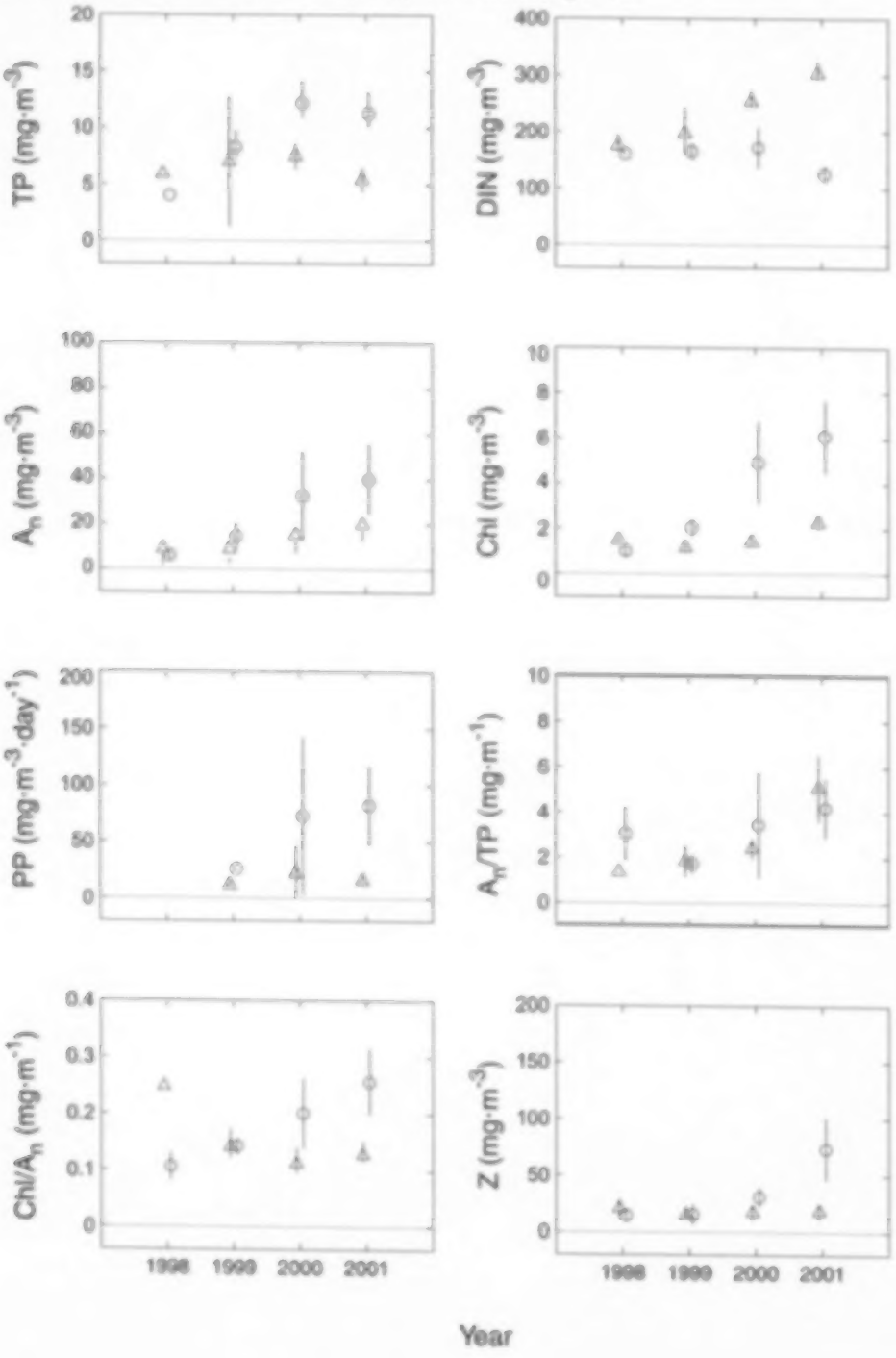

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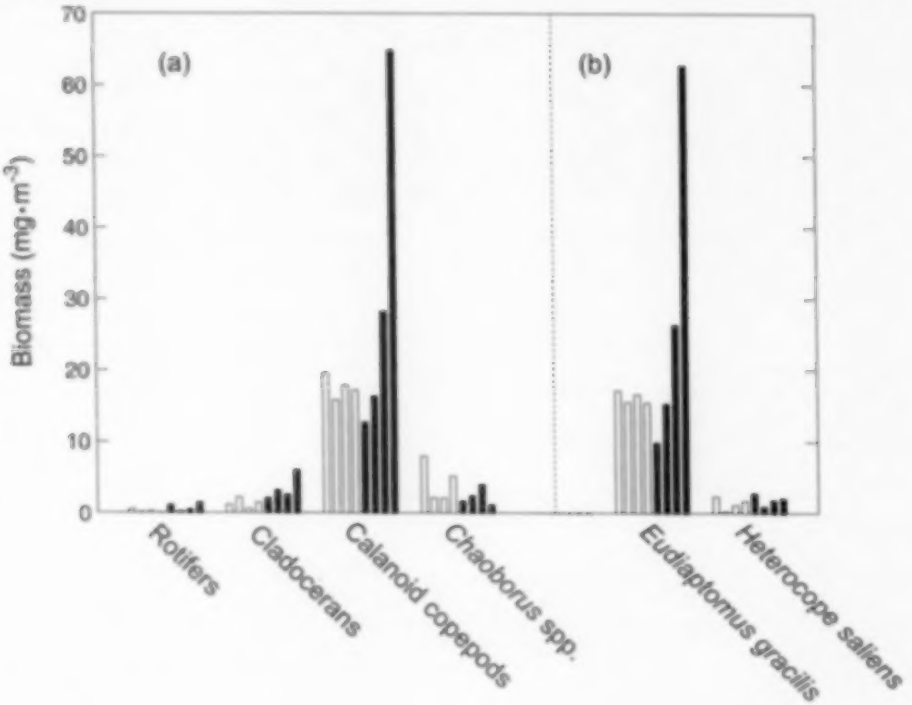
Fig. 4. The taxonomic composition of the zooplankton community: (*a*) composition with respect to main zooplankton groups; (*b*) composition with respect to the two most-dominating zooplankton species. The open and solid bars represent biomass values (carbon) in the reference and treatment basins, respectively. For each, the four bars represents the years 1998, 1999, 2000, and 2001, respectively, when read from left to right.



and the biomass of *D. brachyurum*, the dominant cladoceran, also showed a moderate increase during 1999–2000 and a marked increase in 2001.

Primary production

Fertilization also had a statistically significant effect on primary production (Tables 2 and 3), which increased from 16.5 ± 12.1 mg·m^{-3}·day^{-1} in the reference basin to 58.9 ± 39.1 mg·m^{-3}·day^{-1} in the treatment basin, both as an average for the 3-year fertilization period (Table 2). However, within the 3-year period, the effect of fertilization increased substantially over time (Fig. 3). However, this time trend did not result in any significant interaction term between treatment and year in the ANOVA model.

Sedimentation

The sedimentation rates of POC, PON, and POP were larger in the treatment basin than in the reference basin, as an average for 1999 to 2001 (Table 2). However, the difference between the basins was not significant when tested for POC sedimentation rate (Table 3). Whereas the POC sedimentation rate in the reference basin was 18.9 ± 5.4 mg·m^{-3}·day^{-1} as the average for 1999–2001, the corresponding value for the treatment basin was 26.9 ± 11.3 mg·m^{-3}·day^{-1}. As for the POC sedimentation rate, the relative increases in the sedimentation rates of PON and POP were also small (Table 2). The changes in sedimentation rate of POC, PON, and POP resulted in only slightly decreased C–nutrient ratio in the sedimenting matter in the treatment basin relative to the reference basin, when expressed as the average for all 3 years.

Discussion

As in our study, the lack of replication and the difficulty of having real control treatments are of real concern when doing ecosystem experiments (Schindler 1998). We have based our judgement regarding the effect of fertilization on the comparison of the treatment basin with the reference basin for each of the seasons with fertilization. This approach effectively corrects for between-year variability that is common for both basins but depends on the assumption that the two basins are similar before treatment. The latter assumption is supported by our data from 1998, when no fertilizer was applied, showing the two basins not to be significantly different with respect to any of the measured variables. The general lack of serial-correlated residuals in the ANOVA models is most probably due to our choice of month of the year as one model factor, efficiently accounting for the serial pattern in the data.

Our data clearly indicate that inorganic P addition resulted in substantial basin differences in the epilimnion chemistry. Although POC was unaffected, both PON and POP showed significant increases resulting from fertilization. The stoi-

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chiometry of POM change was close to the Redfield ratio (Redfield 1958). This indicates that the increased POM was of autochthonous origin and not the result of any increased import of particulate matter from the terrestrial surroundings. Hence, the increased levels of PON and POP in the treatment basin are most probably a result of increased nutrient uptake in algae and bacteria, a claim supported by a negative basin difference with respect to NO_3^- and NH_4^+. Moreover, for NO_3^-, the effect of fertilization changed significantly between the 3 years with fertilization, giving rise to a significant interaction term between treatment and year in the model. No significant increases were found in the carbon sedimentation rate.

The observed dominant phytoplankton species are typical of nutrient-poor waters (Findlay and Kasian 1991) and are known to thrive in acid waters as well (Brettum 1992). Also, the original similarity in phytoplankton species composition in the two lake basins is expected because of their similar chemical and morphometrical characteristics. The observed increase in algal biomass resulting from fertilization, without a substantial change in the present species inventory, has also been shown for other lakes receiving moderate P addition (Davison et al. 1995; Blomqvist 1996). As advocated by Davison et al. (1995), this renders the method of moderate P addition favourable relative to liming with respect to conservation issues. Larger P additions usually also change species composition with increased dominance of chlorophytes (Davison et al. 1995) or cyanophytes. In some cases, even moderate P additions conducted to increase fish yields have triggered more pronounced changes in the phytoplankton community (Reinertsen 1982). In our experiment, the P addition did not induce any cyanobacterial outbreak, probably because of low pH (<5.5) and high CO_2 concentrations, as well as moderate total-P concentrations in both basins (Shapiro 1990).

The small response in the nanoalgal biomass to fertilization in 1999 relative to 2000 and 2001 may be due to lower additions of TP in 1999 than in the next 2 years. If this is correct, then the algal P yield, here defined as the nanoalgal carbon biomass per available P unit (A_c/TP), should show minor variation during the same period. However, the variation in P yield during 1998–2001 did not give any clear evidence for this conclusion. Hence, the nanoalgae apparently had a delayed biomass response to fertilization. However, we are not in the position to judge whether this reflects a delayed increase in growth rate or a delayed decrease in loss rates for any of the two phytoplankton groups. Interestingly, we found a steady increase in the P yield in the reference basin during all treatment years, for which we have no explanation.

For both basins, the dominant zooplankton species, as well as the species composition, are typical of acidic lakes in Scandinavia (Stenson et al. 1993). Moreover, the carbon biomass between 13 and 69 mg·m^{-3} is in the lower part of the range reported for humic lakes with similar or somewhat higher P levels (Jansson et al. 2001). Because the water quality in terms of pH and toxic aluminium was not substantially improved during the fertilizations period, it seems reasonable that acid-sensitive species such as *Daphnia* spp. did not appear in the treatment basin.

The almost-exponential increase in zooplankton biomass in the treatment basin during 1998 to 2001 is most likely due to increased food availability, food quality, decreased mortality, or a combination of these factors. Although particulate detritus seemed to remain unchanged after fertilization, the nanoalgae increased substantially, and our data also indicate (see below) that the sum of ciliates and picoplankton increased dramatically. However, because the dominant herbivore in the treatment basin, *E. gracilis*, has been shown to prefer food particles in the 15- to 20-μm size range (Geller and Müller 1981; Hessen 1985; Bern 1994), free-living picoplankton was probably not an important food source for the crustacean zooplankton. Moreover, the nanoalgal biomass peaked earlier than the crustacean biomass during the summer, making a mismatch in nanoalgal availability for zooplankton utilization. This was most pronounced in 2001, between mid-July and mid-September, when the nanoalgal carbon biomass was <10 mg·m^{-3} but the zooplankton biomass exceeded 100 mg·m^{-3} for several weeks. Assuming a maximum per capita production rate of 0.5 day^{-1} for the autotrophs and a minimum per capita respiration loss of 0.20 day^{-1} for the heterotrophs (Andersen 1997), the nanoalgal biomass could possibly not have sustained the zooplankton biomass during its peak period. Hence, our data indicate that because the nanoalgae could not have been the only diet for the zooplankton and free-living picoplankton are not available to the mesozooplankton community because of their small size, ciliates and particulate detritus (Poulet 1983; Pace 1986) are the only remaining particulate food source making up the mezozooplankton diet.

Decreased predation pressure could also contribute to the observed increase in the treatment basin's zooplankton biomass during 1998–2001. In both basins, which were practically devoid of fish populations, the phantom midge *Chaoborus* spp. was the overall dominating predator on crustacean zooplankton. Our data indicate a reduction of the *Chaoborus* spp. population in the treatment basin in 2001 compared with the previous years (data not shown). This may have contributed to the large increase in zooplankton biomass from 2000 to 2001.

Unfortunately, the biomasses of picoalgae, heterotrophic bacteria, and ciliates were not quantified. However, from our data we are able to estimate an upper bound of the sum of these planktonic organisms. The basin difference in POC must be due to the sum of basin differences in the carbon masses of nanoalgae, zooplankton, picoplankton, ciliates, and detritus:

$$\Delta\mathrm{POC} = \Delta A_c + \Delta Z + \Delta(\text{picoplankton} + \text{ciliates}) + \Delta\text{detritus}$$

where Δ denotes the difference between the treatment basin and reference basin. As explained earlier, the stoichiometry of the POM change (ΔPOC:ΔPON:ΔPOP) had a C:N:P ratio of 46:9:1 as an average for the 3 years with fertilization. This stoichiometry is close to that of living plankton (Redfield 1958), indicating that detritus was a minor part of the basin difference in POM. Thus, we assume Δdetritus to be equal to zero. Because ΔA_c and ΔZ are known, we can estimate the basin differences for the sum of ciliate and picoplankton carbon from the equation above. When using

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our time-series data (not shown), we found the basin difference of the sum of ciliates and picoplankton to be 126 ± 52 mg·m^{-3} (mean ± standard error of the mean) for the entire 3-year fertilization period. This implies that the sum of ciliates and picoplankton had a biomass increase resulting from fertilization that was more than six times larger than that of the nanoalgae, as an average for the 3 years with fertilization. Moreover, the Chl/A_p ratio seemed to increase in the treatment basin during all years with fertilization. This could indicate either an adaption to lower light levels owing to increased algal biomass and self-shading or an increased proportion of picoalgae in the phytoplankton community owing to fertilization (Suttle et al. 1991). With respect to light, our Secchi depth measurements do not indicate any decreased light levels in the treatment basin as the result of fertilization (data not shown). Thus, the picoalgae have probably increased in relative abundance in the treatment basin during the fertilization.

Large increases in picoplankton would probably be responsible for taking up much of the added inorganic P, which can explain why only nanoalgal species known to exhibit mixotrophy were able to increase their biomass substantially during fertilization. These chrysophyte species, *D. crenulatum*, *D. sociale* v. *americanum*, *M. allorgei*, and *Ochromonas* spp., probably obtained most of their P by feeding on bacteria or picoalgae and not by direct uptake of inorganic P, whereas the nonmixotrophic algal species have had to compete with the bacteria for inorganic P and therefore have not been able to increase substantially. To what extent benthic or periphytic algae competed with plankton algae for the added P (Mazumder et al. 1989; Axler and Reuter 1996; Wurtsbaugh et al. 2001) is unknown from our study.

As for the nanoalgal biomass response, the response in primary production rate also showed a delayed response to fertilization. The primary production rate increased as a seasonal average from 1999 to 2000 and 2001. This could not be due to the late start of measurements in the first year, when we missed the algal spring bloom. Our time-series data (not shown) showed that the primary production in the treatment basin during 1999 was less than that in 2000 and 2001 also for other parts of the growing season than spring. This observation supports our claim of a delayed response in primary production in the first year of fertilization (time-series data not shown).

In conclusion, the P fertilization resulted in substantial increases in the biomasses of nanoalgae and zooplankton. However, for both groups, only the already-dominant species, which were few in number, increased significantly in biomass, the rest exhibiting minor to modest increases. Thus, the fertilization did not result in any substantial changes in the species inventory, although the biomass composition of the present species changed somewhat. Another striking feature of our data is the systematic delay in response to fertilization. Nanoalgae, zooplankton, and primary production all showed significant increases from 1999 to 2001, the span of the fertilization treatment. Even though much insight has been gained during the last decades on humic lakes, we still know relatively little about the long-term responses of biomass structure to environmental changes and the relative importance of different processes in the carbon turnover. Our findings of delayed biomass responses to fertilization over at least a 3-year period were unexpected and stress the importance of studying the impact of environmental variables on whole-lake scale and over sufficient time spans if relevant insight is to be gained.

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$\delta^{15}N$ in riverine food webs: effects of N inputs from agricultural watersheds

Caroline Anderson and Gilbert Cabana

Abstract: We examined the use of the natural abundance of nitrogen stable isotopes ($\delta^{15}N$) as a tracer of anthropogenic perturbations of the nitrogen (N) cycle at the watershed scale in 82 river sites draining 13 watersheds in the St. Lawrence Lowlands in Quebec. Mean $\delta^{15}N$ values of aquatic primary consumers varied greatly among sites (+2‰ to +15‰), most of this variation (88%) being attributable to site effects. Variation in $\delta^{15}N$ values among functional feeding groups of primary consumers within sites was comparatively lower (<1‰). Within watersheds, $\delta^{15}N$ values of primary consumers (and organisms of higher trophic levels) tracked longitudinal changes in the percentage of agricultural area. Overall, the percentage of total watershed area under agriculture explained up to 69% of the variation in mean primary consumer $\delta^{15}N$ values. Similar positive correlations were observed for predatory invertebrates and non-piscivorous fish. In general, our results show that $\delta^{15}N$ in riverine food webs reacts strongly to spatial patterns in the intensity of N inputs related to agricultural land use.

Résumé : Nous avons évalué l'utilisation de l'abondance naturelle des isotopes stables d'azote ($\delta^{15}N$) comme traceur des perturbations anthropiques du cycle de l'azote (N) à l'échelle du bassin versant dans 82 sites de rivière dans 13 bassins hydrographiques des terres basses du Saint-Laurent au Québec, Canada. La valeur moyenne de $\delta^{15}N$ des consommateurs primaires aquatiques varie considérablement d'un site à un autre (+2 ‰ à +15 ‰), la majorité (88 %) de la variation attribuable aux effets du site. La variation de $\delta^{15}N$ entre les groupes fonctionnels de consommateurs primaires est par comparaison plus faible (<1 ‰). À l'intérieur d'un même bassin versant, les valeurs de $\delta^{15}N$ des consommateurs primaires (et des consommateurs des niveaux supérieurs) suivent les changements longitudinaux dans le pourcentage de terres agricoles. Dans l'ensemble, la pourcentage du bassin versant total utilisé pour l'agriculture explique jusqu'à 69 % de la variation de la valeur moyenne de $\delta^{15}N$ chez les consommateurs primaires. De semblables corrélations positives s'observent aussi chez les invertébrés prédateurs et les poissons non piscivores. En général, nos résultats indiquent que les valeurs de $\delta^{15}N$ dans les réseaux alimentaires des cours d'eau réagissent fortement aux patterns spatiaux d'apports de N reliés à l'utilisation des terres pour l'agriculture.

[Traduit par la Rédaction]

Introduction

Excessive loads of nitrogen (N) to aquatic ecosystems resulting from anthropogenic activity on watersheds (agricultural practices and urban development) have increased the deterioration of many streams, rivers, lakes, and coastal regions globally (Howarth 1998; Rabalais 2002). Negative impacts of surplus N on aquatic systems include shifts in food webs, disruption of ecosystem functioning, and loss of biodiversity (Rabalais 2002). Globally, agricultural practices associated with the human food chain (chemical and natural N fixation, plant growing, meat production) accounted for more than five times the amounts of N released by fossil combustion in the mid-1990s (Galloway and Cowling 2002). Increased dedication of land to agricultural practices has been shown to result in increased N concentration and widespread aquatic-ecosystem degradation (e.g., Harding et al. 1999; Kronvang et al. 1999; Whitehead et al. 2002)

Stable nitrogen isotope ratios ($\delta^{15}N$) of dissolved N, particulate N, and consumers from the food web are increasingly being used as evidence for the incorporation of allochtonous sources of N in freshwater and marine ecosystems (e.g., Cabana and Rasmussen 1996; McClelland et al. 1997; Mayer et al. 2002). Several watershed studies have shown that the high $\delta^{15}N$ value of inorganic N derived from sewage (+10‰ to +25‰; Heaton 1986; Kendall 1998) can be traced in aquatic food webs influenced by urban development (e.g., Cabana and Rasmussen 1996; McClelland and Valiela 1998; Lake et al. 2001). In contrast, other studies have shown how the incorporation of low $\delta^{15}N$ from point-source inputs of particulate matter derived from domestic wastes can result in whole food webs having lower $\delta^{15}N$ in impacted sites than in non-impacted sites (e.g., Van Dover et al. 1992; Tucker et al.

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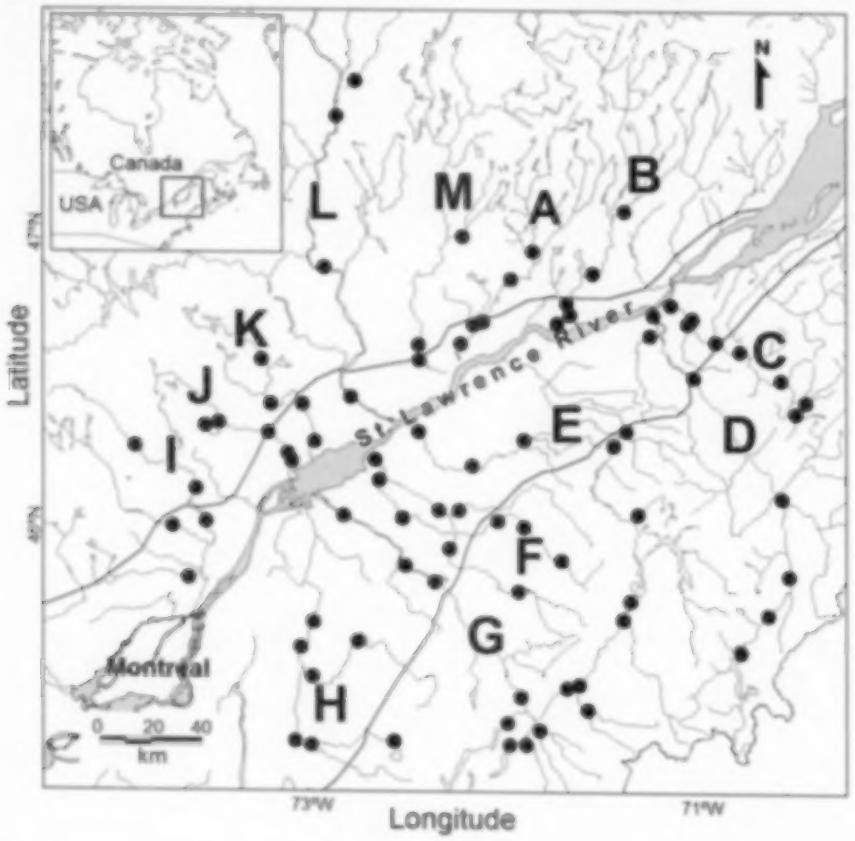
Fig. 1. Watersheds and stations (●) sampled during the summer of 2000. Watersheds are as follows (clockwise): A, Sainte-Anne; B, Jacques-Cartier; C, Etchemin; D, Chaudière; E, Bécancour; F, Nicolet; G, Saint-François; H, Yamaska; I, L'Assomption; J, Maskinongé; K, Du Loup; L, Saint-Maurice; and M, Batiscan. Thicker lines delineate the three physiographic regions: Appalachians (lower section), St. Lawrence Lowlands (middle section), and Canadian Shield (upper section).



1999; DeBruyn and Rasmussen 2002). In agricultural areas little affected by urban development, and where animal wastes are often spread on the land (e.g., Beaulieu 2004), $\delta^{15}N$ in aquatic organisms has been shown to be positively correlated with the percentage of upstream watershed area devoted to agriculture (Harrington et al. 1998; Hebert and Wassenaar 2001; Udy and Bunn 2001). Thus, with some caveats related to the type of anthropogenic N inputs involved (agricultural or domestic wastes), it has been suggested that $\delta^{15}N$ of aquatic food web components as end-points may constitute a useful spatial and temporal integrator of allochtonous N incorporation in developed watersheds. The usefulness of this approach relies first on identifying the importance of food-web processes (trophic-level effects, functional feeding groups) that may influence $\delta^{15}N$ at the local scale and therefore bring into question the ability of this technique to adequately characterize among-site variation in relation to land use. Secondly, the spatial scale of the integration is also an issue, particularly the ability of the technique to detect spatial changes in diffuse or point-source N inputs not only between systems but also within rivers. In this study, we focus on N inputs of agricultural origin. Using a comparative approach, our aims are to (*i*) evaluate within-web effects on $\delta^{15}N$ relative to among-site variation and (*ii*) examine the relationship between $\delta^{15}N$ and agriculture, among and within rivers, using simple whole-watershed descriptions.

Materials and methods

Study sites

During the summer of 2000 (early June to late August), invertebrates and fish were sampled from 82 river stations in the St. Lawrence Lowlands, Quebec (Fig. 1). Study sites were all located in riffle sections within 13 watersheds. The Yamaska (eutrophic; seven sites) and Du Loup (oligotrophic; four sites) river sites were sampled twice (in early June and late August 2000) to examine the temporal robustness of isotopic profiles of invertebrates and small non-piscivorous fish, and no significant difference was found for either river (paired *t* test, $p > 0.05$). Water levels were consistently high throughout the summer of 2000, with lower levels occurring at the end of the summer period. These discharge levels did not differ from the 1980–2000 averages (*z*-score analysis on four rivers from our network, $p < 0.05$).

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Study sites are distributed along three physiographic regions showing different physical and geological characteristics (Dresser and Denis 1946; Dubé 1994). The Canadian Shield and the Appalachians are mostly mountainous regions, while the St. Lawrence Lowlands consist of sedimentary plains. Agriculture is carried out principally in the St. Lawrence Lowlands, especially south of the St. Lawrence River. Watersheds located south of the St. Lawrence River are characterized by important agricultural areas devoted to cropland (mostly corn) cultures requiring large amounts of fertilizers and manure, as well as pasture land. Moreover, these watersheds are characterized by large animal densities, principally pigs and bovines. Conversely, the northern shore is dominated by forests (mostly mixed forests), agriculture being carried out only in the downstream portion of the watersheds, close to the St. Lawrence River. Urban land use occupies less than 5% of the total area of each watershed, the remaining areas not devoted to either agriculture or urban development being dominated by forests.

Sample collection and analysis

Invertebrates and fish were collected with a D-frame aquatic net by kick-sampling (Hauer and Resh 1996). Primary consumers were collected in all 82 sites, while predatory invertebrates were obtained in 76 sites and fish in 65 sites. Up to five functional feeding groups of primary consumers were collected per sampling site (median of three per site) and the median number of species per functional feeding group was 2 (1–6). Samples were kept in coolers and frozen within 8 h and then thawed for sorting and identification to family or genus (invertebrates) or species (fish). Captured fish were adults of small (<12 cm long) non-piscivorous species (see Appendix A).

Once identified, all samples were dried at 60 °C for at least 48 h and ground into a homogeneous powder. Up to 86 individuals were pooled per sample in the case of small abundant organisms such as Gammaridae and Hydropsychidae, while single whole individuals were used for larger predatory invertebrates (e.g., Corydalidae, Perlidae, and Gomphidae). In the case of fish, muscle tissues were analysed. Isotopic analyses were performed at the University of New Brunswick with a Finnigan–Mat Delta Plus mass spectrometer. Isotope ratios were expressed following the standard equation:

$$(1) \qquad \delta^{15}N = [(^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard}) - 1] \times 1000$$

where isotope ratios are expressed as the relative parts per thousand (‰) ratio between a sample and a known reference material, which, by convention, is atmospheric N (N_2). One thousand, two hundred and six samples collected in 2000 (5–34 per site) were analysed and a subset of 93 samples were analysed in duplicate (mean standard deviation 0.17‰). Organisms were classified into trophic groups (predatory or not) and functional feeding groups (scrapers, filter-feeders, shredders, collector–filterers, collector–gatherers) of non-predatory invertebrates according to Merritt and Cummins' (1996) classification of functional feeding groups and to Thorp and Covich's (1991) descriptions. Information on diets of adult fish was obtained from Scott and Crossman (1974).

Land-use analysis

Watersheds in all 82 sites were delineated with ArcInfo® 8.0.2 (ESRI Inc., Redlands, Calif.) and Topaz® 1.20 (Martzl and Garbrecht, developers; http://duke.usask.ca/~martzl/topaz/index.html) using topographic and hydrological georeferenced databases from Natural Resources Canada (National Topographic Data Base; http://www.cits.rncan.gc.ca/cit/servlet/CIT/site_id = 01&page_id = 1-005-002-001.html)

Land-use characterization was done with MapInfo® 5.5 (MapInfo Corporation, Troy, N.Y.) for all 82 watersheds, and information on proportions of agricultural land (area devoted to cropland, pasture, and forage crops) in the watershed, upstream of sampling sites, was derived from databases on dominant land-use types (Natural Resources Canada, http://geogratis.cgdi.gc.ca/CLI/mapping_landuse.html; 1996 Canadian census of Agriculture, Statistics Canada 1998). More detailed land-use information can be obtained in Anderson (2003). Area of subwatersheds was used as a measure of the upstream–downstream position of stations within a watershed.

Statistical analysis

Relationships between $\delta^{15}N$ and land use were examined using analysis of variance (ANOVA) and linear-regression analyses (SYSTAT® version 8.0, SPSS Inc. 1998; SAS® version 8.0, SAS Institute Inc. 1999). Assumptions related to the use of ANOVA were tested using a variance-ratio test (ratio of the largest against the smallest variance; Zar 1999). Arcsine (square root(p)) transformation (Zar 1999) was used in least-squares regression analysis of the percentage of agricultural area on watersheds. Differences in mean $\delta^{15}N$ values among pairs of functional feeding groups within sites were tested with paired t tests using a Bonferroni correction for the number of possible pairwise comparisons ($n = 10$). We examined the presence of spatial autocorrelation among river sites using Moran's I values (see Legendre and Legendre 1998). Moran's I values and their significance were calculated for all pairs of adjacent sites within each river with the Rookcase program (Sawada 1999), and only the sites located on the Du Loup River exhibited significant spatial autocorrelation. To avoid bias related to the presence of spatially autocorrelated sites, only the most downstream sites of all 13 watersheds (independent from one another; $n = 13$) were used in the ANOVAs. In other cases where significant spatial autocorrelation could affect our results, Dutilleul's modified t test was applied (see Legendre et al. 2002).

Results

Intrasite variation in $\delta^{15}N$ values

Within-site pairwise differences in mean $\delta^{15}N$ values among functional feeding groups of primary consumers for all 82 sites ranged from 0.06‰ to 0.91‰ (mean $\Delta\delta^{15}N$ = 0.43‰) and although some of these were statistically significant for certain pairs of functional feeding groups (paired t tests; Table 1), the differences remained relatively small (<1‰), therefore justifying the pooling of all functional feeding groups by trophic level in subsequent analyses. The slopes of the relationships of $\delta^{15}N$ values among functional feeding groups were all highly significant ($r^2 > 0.77$, $p <$

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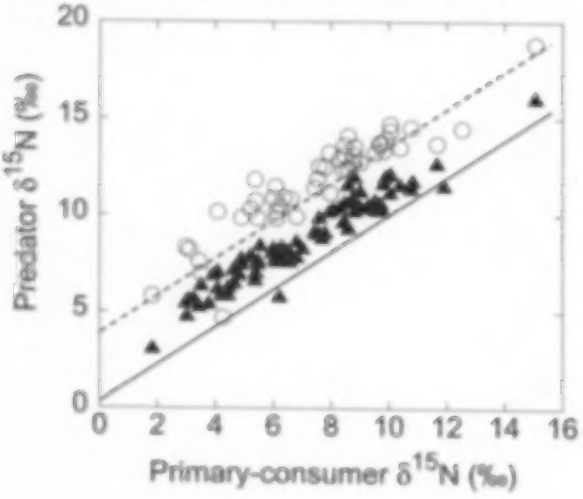
Table 1. Average within-site differences in $\delta^{15}N$ values ($\Delta\delta^{15}N$) between invertebrate functional feeding groups (lower left side of the matrix; numbers in parentheses show the number of sites) and slopes and intercepts of the across-site $\delta^{15}N$–$\delta^{15}N$ relationships between invertebrate functional feeding groups (upper right).

Functional feeding group	Collector–filterer	Gatherer	Scraper	Detritivore	Filter-feeder
Collector–filterer	—	1.1, –0.3	1.0, 0.6	0.9, 1.7	1.1, 0.1
Gatherer	–0.06 (43)	—	1.0, 0.4	0.8, 2.1	1.1, –0.9
Scraper	**–0.55** (71)	**–0.38** (34)	—	0.8, 1.4	1.0, 0.0
Detritivore	**–0.88** (50)	**–0.91**(24)	–0.31 (45)	—	1.0, 0.5
Filter-feeder	**–0.83** (20)	–0.34 (9)	–0.07 (17)	–0.09 (16)	—

Note: The significance of differences in mean $\delta^{15}N$ values of functional feeding groups ($\Delta\delta^{15}N$) was determined with paired t tests (Bonferroni-corrected). Values that are significantly different ($p < 0.05$) are shown in boldface type. Isotopic enrichment is presented as the enrichment of groups in a row compared with groups in a column. Slopes (b) and intercepts (a) for the relationship $\delta^{15}N(y) = a + b\delta^{15}N(x)$ are presented for groups in rows versus groups in columns.

Fig. 2. Relationships between mean $\delta^{15}N$ values of predatory invertebrates (▲; regression line: $y = 2.7 + 0.9x$; $r^2 = 0.92$, $p < 0.0001$, $n = 76$) and non-piscivorous fish (○; $y = 5.4 + 0.9x$; $r^2 = 0.86$, $p < 0.0001$, $n = 65$) and mean $\delta^{15}N$ values of primary consumers for sites sampled in 2000. The solid line represents the 1:1 relationship and the broken line corresponds to the fractionation factor expected for secondary consumers compared with primary consumers (+3.4‰; Minagawa and Wada 1984).



0.0001, for all possible relationships in Table 1), suggesting that $\delta^{15}N$ values of all groups varied similarly among sites.

Mean $\delta^{15}N$ values of primary consumers collected in 2000 varied greatly among sites (+2‰ to +15‰), with sites accounting for 88% of the total variation (one-way ANOVA on the 13 most downstream sites; site effects: $F_{[12,83]} = 50.5$, $p < 0.0001$). Thus, much of the variance in $\delta^{15}N$ values was spatial rather than being accounted for by variation among species of primary consumers within sites. The $\delta^{15}N$ values of predatory invertebrates and fish were highly correlated with the $\delta^{15}N$ values of primary consumers across sites (Fig. 2), both groups of secondary consumers being enriched in ^{15}N relative to primary consumers (mean $\Delta\delta^{15}N = +1.8‰$, standard error (SE) = 0.1‰, $n = 76$, for predatory invertebrates and mean $\Delta\delta^{15}N = +4.3‰$, SE = 0.1‰, $n = 65$, for fish).

Fig. 3. Relationships between $\delta^{15}N$ values of primary consumers (regression line: $y = 4.2 + 0.1x$) (*a*), predatory invertebrates ($y = 6.3 + 0.1x$) (*b*), and fish ($y = 9.0 + 0.1x$) (*c*) and the percentage of agricultural land in watersheds located upstream of sampling sites. The most downstream sites of each watershed are indicated (●). The solid line represents least squares regression lines for all sites combined.

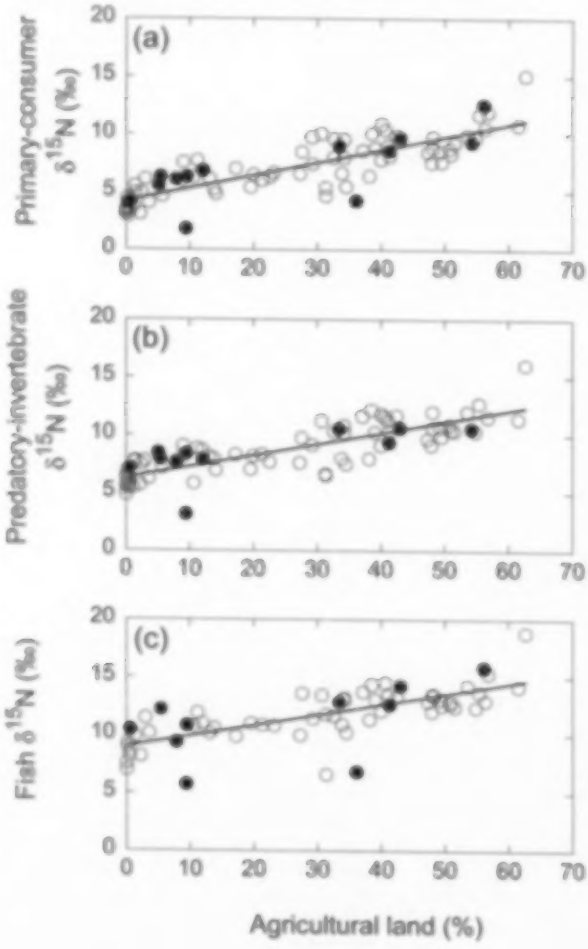


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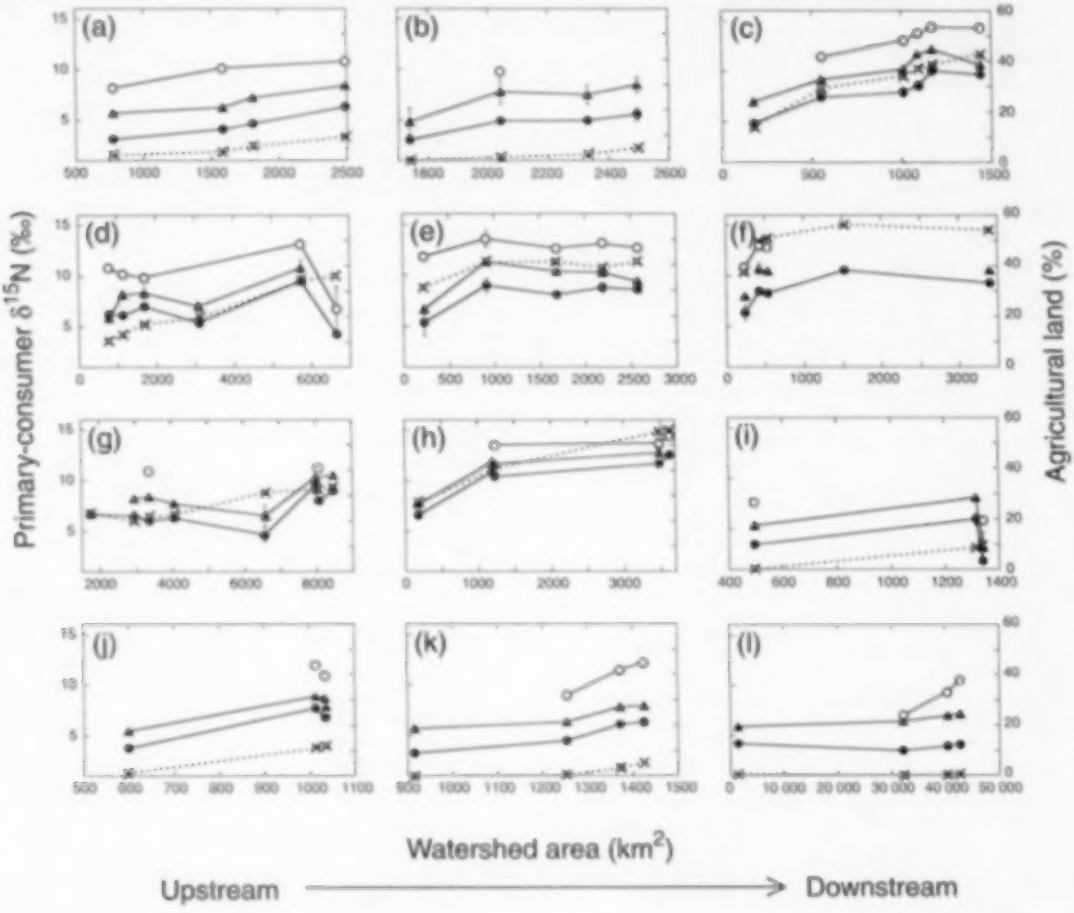
Fig. 4. Longitudinal relationships between $\delta^{15}N$ values of primary consumers (●), predatory invertebrates (▲), fish (○), and percentage of agricultural land (×) and watershed area for the Sainte-Anne (*a*), Jacques-Cartier (*b*), Etchemin (*c*), Chaudière (*d*), Bécancour (*e*), Nicolet (*f*), Saint-François (*g*), Yamaska (*h*), L'Assomption (*i*), Maskinongé (*j*), Du Loup (*k*), and Saint-Maurice (*l*) rivers. For each site, watershed area is a measure of longitudinal position along the watershed. Note that only sites located on main river stems (following each other longitudinally) were used in this analysis ($n = 69$). Error bars correspond to ±1 standard error.



Mean $\delta^{15}N$ values of aquatic primary consumers collected in downstream river sites showed a significant positive relationship with the percentage of agricultural area in the watershed (Fig. 3*a*; $r^2 = 0.55$, $p < 0.01$, $n = 13$; equation of regression line: $y = 4.4 + 0.1x$). Predatory invertebrates' $\delta^{15}N$ values also increased in parallel with the percentage of agricultural area (Fig. 3*b*), although the proportions of the variance explained by the linear-regression model were slightly lower than those obtained with primary consumers ($r^2 = 0.43$, $p < 0.05$, $n = 11$; $y = 6.8 + 0.1x$). Conversely, fish $\delta^{15}N$ values did not seem to be significantly correlated with the percentage of agricultural area in downstream sites ($p > 0.05$). However, when all study sites were used, $\delta^{15}N$ values for primary consumers ($r^2 = 0.69$, $n = 82$), predatory invertebrates ($r^2 = 0.65$, $n = 76$), and fish ($r^2 = 0.52$, $n = 65$) were all highly correlated with the percentage of agricultural area. These relationships were all highly significant ($p < 0.0001$), even when they were examined with Dutilleul's modified *t* test, which reduces the degrees of freedom.

Longitudinal variation in $\delta^{15}N$ values within rivers

Within rivers, mean $\delta^{15}N$ values of primary consumers increased progressively or remained constant from upstream to downstream sites and, with few exceptions, followed agricultural development (Fig. 4). Ten of 12 rivers showed increases in area devoted to agriculture going downstream, and in 5 of these rivers, primary consumers' $\delta^{15}N$ values increased significantly with agricultural area (*t* test on least-squares regression slopes, $p < 0.05$). The Du Loup River also showed this trend, but the use of Dutilleul's modified *t* test reduced the degrees of freedom such that the relationship was not significant ($p > 0.05$). Furthermore, two rivers (Figs. 4*e*, 4*f*) showed no significant increases in the percentage of agricultural area with increasing watershed area ($p >$

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0.05), and in those watersheds, $\delta^{15}N$ values of primary consumers remained constant. In general, variation in $\delta^{15}N$ seems to be correlated with the percentage of agricultural area, both variables tending to increase from upstream to downstream sites or remaining constant simultaneously. However, some rivers did not show this trend, $\delta^{15}N$ decreasing either slightly (Figs. 4*g*, 4*j*) or sharply (Figs. 4*d*, 4*i*) as the percentage of agricultural area continued to increase.

Discussion

Intrasite variation in $\delta^{15}N$ values among functional feeding and trophic groups

Significant but small (<1‰, compared with among-site variation of 13‰) intrasite variation in $\delta^{15}N$ was found among functional feeding groups of primary consumers in 2000. In particular, $\delta^{15}N$ of collector–filterers was significantly enriched compared with that of detritivores, filter-feeders, and scrapers, while gatherer–collectors were enriched compared with scrapers and detritivores. Collectors' ^{15}N enrichment could be linked to ingestion of animal food, thus increasing their trophic position. Such ingestion of animal food by non-predatory invertebrates has been observed (Cummins 1973; Anderson 1976; Parker and Voshell 1983). Zah et al. (2001) observed variability in $\delta^{15}N$ values of non-predatory invertebrates in a glacier stream. They found that some sites showed overlap in $\delta^{15}N$ values of primary consumers and predators, which was related to the high $\delta^{15}N$ values of gatherer–collectors. They suggested that omnivory was the origin of higher $\delta^{15}N$ values in gatherer–collectors, an observation that is consistent with our findings suggesting that collectors might occupy slightly higher trophic levels. Moreover, omnivory in predatory invertebrates, as well as physiological effects on fractionation related to an invertebrate diet (McCutchan et al. 2003), might also contribute to the weaker fractionation factor (less than 3.4‰; Minagawa and Wada 1984) observed between predatory invertebrates and primary consumers. Our results also confirmed those of Vander Zanden and Rasmussen (2001), who observed only slight variation in fractionation between carnivores (a high r^2 value between the $\delta^{15}N$ values of predators and the average $\delta^{15}N$ values of potential prey). Overall, our results show that the functional feeding groups' effects remain small relative to among-site variation, thus supporting the general use of $\delta^{15}N$ values of primary consumers as landscape integrators.

Spatial variation in $\delta^{15}N$ values

The $\delta^{15}N$ of aquatic primary consumers, predatory invertebrates, and fish was strongly related to agricultural development on the watershed. Within most rivers, $\delta^{15}N$ tended to increase from upstream to downstream sites with increasing percentage of agricultural area. In contrast, $\delta^{15}N$ showed little or no longitudinal increases in rivers not showing longitudinal increases in agriculture. Combining all rivers, $\delta^{15}N$ still showed a strong correlation with percentage of agricultural area.

The increased $\delta^{15}N$ values observed in aquatic food webs affected by agriculture can be potentially linked to three factors: (1) variation in the isotopic signal of N sources applied to the fields, (2) biogeochemical N transformations occurring during transport to the river, and (3) in-stream N-transformation processes. Average $\delta^{15}N$ values of industrial fertilizers and freshly produced animal wastes (~0‰ and +5‰ for fertilizers and manure, respectively; Heaton 1986; Kendall 1998) appear to be insufficient by themselves to explain the wide variation in consumer $\delta^{15}N$ values observed in the present study (+2‰ to +15‰ for primary consumers). However, N transformations (e.g., volatilization and denitrification) occurring after N application and leading to gaseous losses are well known to result in further elevation of the remaining unreacted N in ground waters and surface waters (averages of +8.8‰ for fertilizers and +10‰ to +25‰ for animal manure; Kreitler 1979; Heaton 1986; Kendall 1998). Högberg (1990) also showed that increasing amounts of low-$\delta^{15}N$ synthesized fertilizers applied to a field resulted in important increases (up to 11‰) in the $\delta^{15}N$ of terrestrial vegetation. Thus, the wide range in $\delta^{15}N$ values observed here in impacted aquatic organisms could be due to such $\delta^{15}N$-increasing N transformations occurring on the watershed. N-budget calculations (Anderson 2003) for the watersheds studied here showed that applications of N as animal manure exceeded applications as industrially produced fertilizers, suggesting that both the slightly higher $\delta^{15}N$ source signal of manure (compared with that of fertilizers) and the effects of processes leading to $\delta^{15}N$ increases are likely to be responsible for the elevated $\delta^{15}N$ values observed in many sites. Lastly, in-stream nitrification, denitrification, or assimilation of N could also have an influence on the $\delta^{15}N$ values measured (Battaglin et al. 2001; Kellman and Hillaire-Marcel 1998; Kendall 1998). While in-stream denitrification may further increase the $\delta^{15}N$ values of aquatic food webs, assimilation might have the opposite effect, since plants preferably assimilate the lighter isotope (^{14}N) when N is not limiting and could therefore acquire a lower isotopic signal (compared with the $\delta^{15}N$ of inorganic N) in N-enriched rivers (Kendall 1998). Future work involving concurrent $\delta^{15}N$ measurements of inorganic N in water and food-web components could contribute to evaluating the importance of these potential effects in the field.

A few sites dominated by agriculture did not show high $\delta^{15}N$ values. These sites were located downstream of important urban areas and were affected by discharges of untreated industrial or domestic particulate organic matter (e.g., L'Assomption and Chaudière Rivers; Anderson 2003). In contrast to the high $\delta^{15}N$ values observed in inorganic N in eutrophied rivers (e.g., Mayer et al. 2002), low $\delta^{15}N$ values (–2.8‰ to +1.1‰), which have been observed in particulate organic matter collected from urban effluents (Van Dover et al. 1992; Tucker et al. 1999; DeBruyn and Rasmussen 2002), may explain these low residual values.

Overall, these results suggest that in the absence of important point-source N inputs, $\delta^{15}N$ in aquatic organisms will increase in a predictable way, following the increasing amounts of agricultural N inputs. Comparable relationships between $\delta^{15}N$ of indicator organisms and agricultural land use have been observed in previous studies (Harrington et al. 1998; Hebert and Wassenaar 2001; Udy and Bunn 2001). Our results complement these studies, as longitudinal trends within watersheds can help us to better understand how progressive changes in land use affect $\delta^{15}N$. Furthermore, the use of organisms of different trophic levels may help to distinguish those components of the food web that are affected

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by anthropogenic activities from those that avoid them through migration. For instance, the lower correlation between fish $\delta^{15}N$ values and agriculture could be associated with the ability of fish to migrate between watershed sections impacted by various land uses.

In conclusion, our results showed that $\delta^{15}N$ of primary consumers and organisms at higher trophic levels reacted strongly to spatial patterns in the intensity of N inputs associated with agricultural land use within and among watersheds. Furthermore, they reveal that within-site variation in $\delta^{15}N$ among functional feeding groups remained small compared with among-site variation, thus supporting the general use of $\delta^{15}N$ values of primary consumers as landscape integrators of the impact of land use on aquatic ecosystems.

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Appendix A.

Table A1. Classification of fish collected during the summer of 2000; the principal food ingested was algae and detritus (H) or invertebrates (I) (Scott and Crossman 1974).

Species	Feeding group
Catostomus commersoni	I
Cottus bairdi	I
Cottus cognatus	I
Etheostoma flabellare	I
Etheostoma nigrum	I, H
Exoglossum maxillingua	I
Fundulus diaphanus	I
Hybognathus nuchalis	H
Lepomis gibbosus	I
Notropis bifrenatus	I
Notropis volucellus	I, H
Noturus flavus	I, H
Percina caprodes	I
Percina copelandi	I, H
Pimephales notatus	H, I
Rhinichthys atratulus	I
Rhinichthys cataractae	I
Semotilus atromaculatus	I, H

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Linkages between weather, dissolved organic carbon, and cold-water habitat in a Boreal Shield lake recovering from acidification

W. (Bill) Keller, Jocelyne Heneberry and Julie Leduc

Abstract: To investigate potential effects of climate change on lake thermal structure, we examined relationships between the amount of cold-water habitat in late summer (defined as the 10 °C depth), summer weather, and dissolved organic carbon (DOC) concentration over a two-decade period (1981–2002) in a small Boreal Shield lake recovering from acidification. DOC concentration, wind-days (the product of mean daily wind speed and the number of days between ice-out and late-summer stratification), and mean daily temperature were significant predictors of the 10 °C depth in a multiple-regression model. A similar model using simply the number of ice-free days instead of wind-days was almost as effective. The models were quite successful in explaining interannual variations in the 10 °C depth when tested on a chemically and morphometrically similar nearby lake. While factors related to summer weather were important in explaining interannual variations in the amount of late-summer cold-water habitat, increased DOC concentration over the study period largely explained observed long-term decreases in the 10 °C depth (increases in cold-water habitat). DOC concentration was positively correlated with pH. In acidified regions, increases in DOC that accompany the recovery of acidified lakes will need to be considered in assessments of potential climate-change effects on lake thermal structure.

Résumé : Afin d'étudier les effets potentiels du changement climatique sur la structure thermique des lacs, nous avons examiné les relations entre l'importante des habitats d'eau froide à la fin de l'été (délimités par la profondeur du 10 °C), les conditions climatiques estivales et les concentrations de carbone organique dissous (DOC) sur une période de deux décennies (1981–2002) dans un petit lac du bouclier boréal en phase de récupération de l'acidification. La concentration de DOC, les journées de vent (le produit de la vitesse moyenne journalière du vent par le nombre de jours entre la disparition de la glace et la stratification de fin d'été) et les températures journalières moyennes sont toutes des variables prédictives significatives de la profondeur du 10 °C dans un modèle de régression multiple. Un modèle semblable qui n'utilise que le nombre de jours sans glace au lieu du nombre de jours de vent est presque aussi efficace. Les modèles réussissent très bien à expliquer les variations interannuelles de la profondeur du 10 °C lorsqu'on les utilise sur un lac adjacent semblable par sa chimie et sa morphologie. Les facteurs reliés aux conditions climatiques de l'été sont importants pour expliquer les variations d'une année à l'autre de l'étendue des habitats d'eau froide en fin d'été; en revanche, l'accroissement de DOC durant la période d'étude explique en grande partie la réduction à long terme de la profondeur du 10 °C (donc l'augmentation des habitats d'eau froide). La concentration de DOC est en corrélation positive avec le pH. Dans les régions acidifiées, on devra tenir compte de l'accroissement de DOC qui accompagne la récupération des lacs acidifiés lorsqu'on évalue les effets potentiels du changement climatique sur la structure thermique d'un lac.

[Traduit par la Rédaction]

Introduction

Climate change has large implications for aquatic ecosystems (Schindler 2001). The predicted consequences of climate warming for lakes include deepening of the surface mixed layer and losses of cold-water habitat (Fee et al. 1996; Schindler et al. 1996; Schindler 2001), changes that may have important effects on aquatic biota. Changes to lakes could be a direct consequence of changing weather conditions such as increased air temperature or wind speed (Robertson and Ragotzkie 1990; Schindler et al. 1990) or an indirect effect of increased water transparency related to warmer, drier conditions resulting in reduced dissolved organic carbon (DOC) export from lake watersheds (Fee et al. 1996; Schindler et al. 1996; Snucins and Gunn 2000). Alternately, predicted increases in exports of DOC from water-

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Fig. 1. Locations of Whitepine Lake and Aurora Whitepine Lake in northern Ontario.

sheds with increasing atmospheric CO_2 and associated climate change (Clair et al. 1999) suggest that lake transparency could decrease.

While the changes that will occur with a changing climate are uncertain, analyses of existing long-term data series that span periods of varying weather conditions can serve as important guides to the development of reasonable predictions (Schindler et al. 1990). In this paper we examine a two-decade (1981–2002) record of thermal conditions in Whitepine Lake, 89 km north of Sudbury, Ontario (47°17′N, 80°50′W), for evidence of links between the amount of cold-water habitat, water transparency as measured by DOC concentration, and weather. The study period spans a wide range of weather conditions, during which, for example, mean annual air temperatures varied by 3.7 °C, from 0.9 to 4.6 °C. In particular, we examine the year-to-year variability in the late-summer 10 °C depth (an approximation of the upper boundary of cold-water habitat; Snucins and Gunn 2000; Clark et al. 2004) in Whitepine Lake. We then construct multiple linear regression models to attempt to explain this variability in terms of DOC concentration, length of the ice-free period before late-summer stratification (ice-free days), and weather conditions during that period, and test these models on data for a morphometrically similar lake (Aurora Whitepine Lake; 47°23′N, 80°38′W) in the same general area. Cold-water habitat is a very important attribute of many Boreal Shield lakes, particularly those with lake trout (*Salvelinus namaycush*) (Clark et al. 2004). If changes in the amount of cold-water habitat result from climate change, they are expected to have direct effects on cold-water fish and invertebrate species, and also indirect effects on aquatic communities through altered predatory and competitive interactions (Schindler 2001).

Most models explaining attributes of lake thermal structure have been developed for groups of lakes (e.g., Fee et al. 1996; Pérez-Fuentetaja et al. 1999; Snucins and Gunn 2000), where aspects of lake morphometry have important roles in explaining variability in thermal structure. However, by focusing on a single lake subjected to variations in weather and lake transparency through time, the influence of lake morphometry is held constant (e.g., Yan 1983). This allows a direct examination of the relationships between weather-related factors, DOC concentration, and thermal changes, providing a realistic picture of how individual lakes can be expected to respond to changes in weather.

Whitepine Lake (area 66.9 ha, mean depth 5.9 m, maximum depth 22.0 m) is a dilute, oligotrophic (2002 ice-free period averages: conductivity 22.8 $\mu S \cdot cm^{-1}$, total phosphorus 5.6 $\mu g \cdot L^{-1}$, Secchi transparency 5.7 m, chlorophyll *a* concentration 2.0 $\mu g \cdot L^{-1}$) dimictic lake (Fig. 1). Aurora Whitepine Lake (Fig. 1) is morphometrically and chemically similar to Whitepine Lake (area 77.8 ha, mean depth 7.0 m, maximum depth 21.3 m; 2002 ice-free period averages: conductivity 22.7 $\mu S \cdot cm^{-1}$, total phosphorus 4.5 $\mu g \cdot L^{-1}$, Secchi transparency 4.9 m, chlorophyll *a* concentration 1.5 $\mu g \cdot L^{-1}$). Both lakes were acidified by emissions of sulphur from the Sudbury metal smelters, and by sulphur deposition from other sources via long-range transport. With the large reduc-

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tions in sulphur emissions that have been implemented at Sudbury and elsewhere in recent decades, Whitepine Lake, like many other lakes in northeastern Ontario, is recovering from acidification, both chemically and biologically (Gunn and Keller 1990; Keller et al. 2002). Since 1997, the average pH of Whitepine Lake has been above 6.0, while in 1980, when the lake monitoring program started, it was <5.5. Aurora Whitepine Lake, while also recovering from acidification, is still quite acidic (pH 5.1 in 2002). The fact that these lakes are recovering from acidification does not diminish their value as sites to examine potential climate-change effects. Recovery of lakes from acidification is a widespread pattern in northeastern Ontario (Keller et al. 2001, 2003) and is expected to become more common in North America as a result of recently implemented sulphur emission control programs. The responses of many lakes in North America to climate change will be superimposed on, and linked to, their recovery from acidification. Thus, recovery from acidification is an important background against which to examine potential climate-change effects on lakes.

Methods

Whitepine Lake has been sampled approximately monthly or more frequently during the ice-free season since 1980. Aurora Whitepine Lake was sampled on a similar schedule from 1987 to 2002, with the exception of 1989. The pH values (laboratory meter measurements) and DOC concentrations presented here represent non-volume-weighted composite samples through the epilimnion and metalimnion taken with a weighted plastic tube sampler. In the laboratory, particulates in the DOC samples were allowed to settle before aliquots were removed for analysis. Organic carbon was measured when the supernatant of the settled sample was introduced into an AutoAnalyzer system, where it was acidified and flushed with nitrogen at 500 mL·min^{-1} to remove inorganic carbon. The sample was then oxidized with an ultraviolet digester in an acid–persulphate medium. The carbon was converted to carbon dioxide and, following dialysis, was measured by determining the loss in absorbance of a weakly buffered alkaline phenolphthalein solution using a colourimeter operating in the inverse mode.

Temperature profiles were measured at 1-m intervals from surface to 1 m above bottom with electronic probes (accuracy ±0.5 °C). The late-summer profiles used for model development were selected as those from the closest date to 31 August that was available in each year. These dates ranged from 12 August to 11 September. To examine the influence of varying sampling dates, we also employed a database for temperature profiles from an overlapping (71% of the sampling dates) but slightly different late-summer period (5–31 August). Suitable profiles were not available for Whitepine Lake in 1980 and 1995, so these years could not be used in the data analyses.

Air temperatures and wind speeds were obtained from the Environment Canada weather-monitoring site at Earlton Airport, 90 km from Whitepine Lake. Sunshine data were obtained from Kapuskasing Airport, 265 km distant. We used the number of ice-free days in the period before the late-summer sampling date in each year and mean daily values

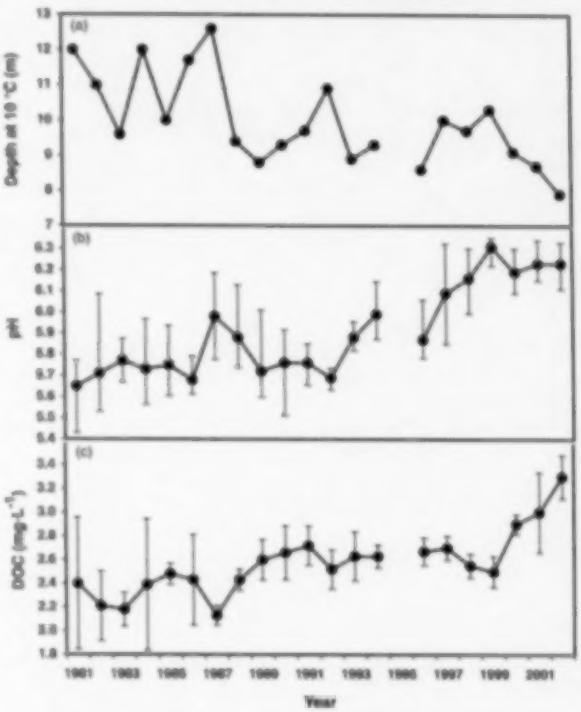

Fig. 2. Depth at 10 °C during late-summer stratification (*a*), mean annual pH (*b*), and dissolved organic carbon (DOC) concentration (*c*) for the ice-out to late summer stratification periods in 1981–2002 for Whitepine Lake. The error bars are ranges for pH and standard deviations for DOC concentration, from monthly values during the ice-out to late summer period.

for weather variables during those periods in our regression modeling. Actual observed ice-out dates were available for only 3 years on Whitepine Lake, therefore we estimated other ice-out dates based on the average difference (12 days) between Whitepine Lake and a lake in the City of Sudbury (Ramsey Lake) with long-term records of ice clearance. We used the same ice-out dates for Aurora Whitepine Lake, since the two lakes are morphometrically similar and only 19 km apart. Ice-out dates ranged from 26 April to 22 May. Data were examined for normality (Kolmogorov–Smirnov test) and transformations were not required. Comparisons of results for regressions based on linear relationships and other forms (logarithmic, inverse, exponential) indicated that over our relatively narrow range of values, patterns between variables were well described by linear models and the use of alternative models was not warranted. Pearson correlations were used to test relationships between variables. Time trends in variables over our study period were examined with the Mann–Kendall test (Hamed and Rao 1998), which accounts for autocorrelation in time series.

Results

The late-summer 10 °C depth in Whitepine Lake showed wide interannual variation over the study period, ranging from 7.9 to 12.6 m (Fig. 2). Annual mean pH (5.7–6.3) and

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DOC concentration (2.1–3.3 mg·L^{-1}) during the ice-out to late-summer stratification period (ice-free days) in Whitepine Lake ranged widely among years (Fig. 2), and pH and DOC concentration were positively correlated ($r = 0.55$, $p < 0.05$). The mean daily weather variables examined for the ice-free periods before late-summer stratification (Fig. 3) also showed interannual variation (air temperature 14.0–17.1 °C, hours of bright sunshine 6.2–8.3, wind speed 10.9–14.9 km·h^{-1}). The only intercorrelation among the three weather variables examined was between air temperature and sunshine ($r = 0.52$, $p < 0.05$). None of the mean weather variables were correlated with the number of ice-free days (range 96–136).

Analysis of time trends for all the above lake and weather variables showed significant ($p < 0.05$) overall increases in pH, DOC concentration, and mean daily air temperature, and a significant decrease in 10 °C depth. No significant temporal trends were detected in other weather variables.

A stepwise multiple linear regression equation for 10 °C depth (eq. 1) was derived from testing DOC concentration, number of ice-free days, and the three mean daily weather variables as predictors and includes only variables with significant ($p < 0.05$) beta coefficients. With this restrictive approach to inclusion of variables, DOC concentration (mg·L^{-1}) number of ice-free days, and mean daily air temperature (°C) were selected as significant ($p < 0.05$, $n = 21$) predictors of depth of 10 °C (m). None of these variables were intercorrelated.

(1) Depth at 10 °C = −2.697(DOC) + 0.048(ice-free days) − 0.460(mean daily air temperature) + 18.687

($R^2 = 0.79$)

Wind did not enter the equation, likely because the effect of wind (and potentially other weather variables) is partially captured by the number of ice-free days, which reflects the time available for wind mixing to affect thermal structure. Using wind-days (the product of mean daily wind speed and number of ice-free days) instead of simply the number of ice-free days in an attempt to better include the wind effect gave a multiple regression that did slightly increase the explained variance. This equation was used in subsequent data analyses.

(2) Depth at 10 °C = −2.999(DOC) + 0.003(wind-days) − 0.373(mean daily air temperature) + 19.224

($R^2 = 0.83$)

Estimates of the relative effects of individual predictor variables on estimates of 10 °C depth were made by manipulating the multiple regression equation including wind-days. The 10 °C depth was predicted using mean values for all the predictor variables for the study period. The annual values of each predictor variable used to develop the model were then used in turn, while the others were held constant at the mean for the study period, to determine relative annual changes in predictions of 10 °C depth. DOC concentration, wind-days, and mean daily air temperature had the greatest effect on predictions for 9, 9, and 3 years, respectively. Using study period means as a base case, it was predicted that changes of 0.33 mg·L^{-1} DOC, 2.7 °C mean summer air temperature, or 344 wind-days (or 21 ice-free days) would change the 10 °C depth by 1 m.

The model developed for Whitepine Lake was successful in explaining much of the interannual variation in the 10 °C depth over the 15-year record for Aurora Whitepine Lake, based on a comparison of predicted and observed values ($r^2 = 0.66$, $p < 0.05$). With the exclusion of one year, 1990, that appeared to be an outlier, the explained variation increased substantially ($r^2 = 0.85$, $p < 0.05$; Fig. 4).

In Whitepine Lake, the model developed using data collected for dates between 12 August and 11 September was also quite successful ($r^2 = 0.64$, $p < 0.05$) in predicting the 10 °C depth when applied to data for dates from a slightly different but overlapping period of summer stratification, 5–31 August.

Discussion

Over the two-decade(1981–2002) record on Whitepine Lake, the wide interannual variability in 10 °C depth (4.7 m) could be explained largely by variations in water transparency (DOC concentration), wind-days (or alternatively simply the number of ice-free days), and mean air temperature. These variables entered significantly ($p < 0.05$) in multiple linear regression equations derived for 10 °C depth. Independently, DOC concentration was the best correlate ($p < 0.05$) with 10 °C depth ($r = -0.72$). The fact that variations in the time available for weather-related variables to operate (number of ice-free days, or wind-days), not simply the variations in the weather variables themselves, were important in explaining variations in late-summer cold-water habitat has important implications. Large-scale regional changes such as longer ice-free seasons, an expected consequence of climate warming (Magnuson et al. 2000), will clearly have important direct effects on lake thermal structure.

A simple sensitivity analysis of the multiple-regression models suggested that both weather and lake transparency can be important in determining thermal structure over the ranges observed in our study. Of course, these factors do not operate independently in the environment, thus our very simple approach to sensitivity analysis only estimates relative potential effects, based on the degree of weather fluctuations observed during our study period.

Our results agree with results from spatial surveys indicating that DOC has an important effect on late-summer ther-

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Fig. 3. Mean daily air temperature (*a*), hours of bright sunshine (*b*), and wind speed (*c*) for the ice-out to late summer stratification periods in 1981–2002 for Whitepine Lake.

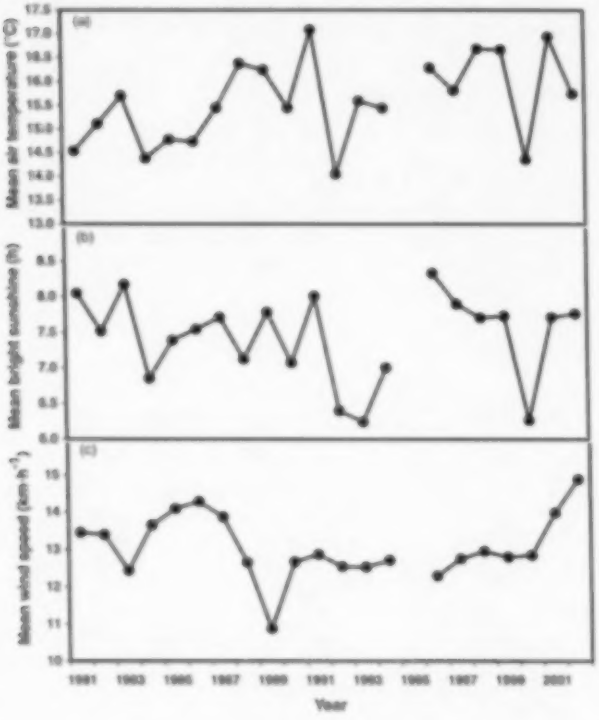
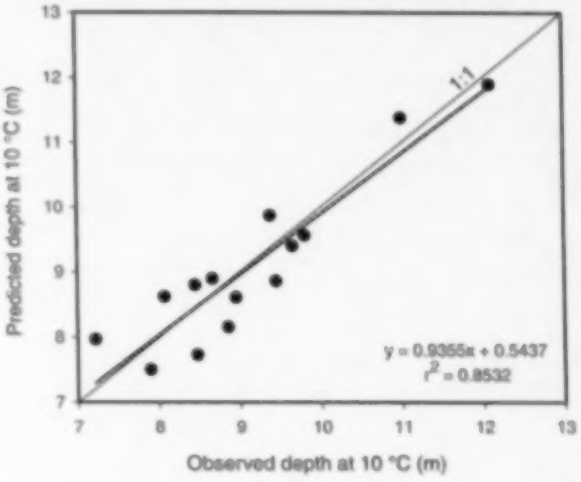


Fig. 4. Comparison of observed and predicted values for the depth at 10 °C in Aurora Whitepine Lake, excluding one outlier, 1990 (n = 14). Values were predicted for Aurora Whitepine Lake using the model developed for Whitepine Lake, including DOC concentration, wind-days, and mean air temperature (eq. 2).



mal structure in small Boreal Shield lakes (Pérez-Fuentetaja et al. 1999; Snucins and Gunn 2000). While weather-related factors were important in explaining annual variations in the cold-water habitat of Whitepine Lake, increased DOC was particularly important in explaining the long-term temporal trend toward decreased 10 °C depth (increased cold-water habitat).

In a number of ways, however, our data are imprecise. Our late-summer sampling dates varied by 1 month. In part these variations are related to sampling logistics, which determined our actual late-summer sampling dates on these fly-in, wilderness lakes. Nevertheless, our model was successful in predicting the 10 °C depth over a slightly different but overlapping period of late-summer stratification for Whitepine Lake, suggesting that variation in sampling dates did not greatly affect our overall results.

Our analyses employed regional weather data from sites distant from the lake, certainly not capturing local variations that may be very important. In a comparison of records for the weather stations we used and records for Sudbury, approximately 170 km to the southwest, mean summer (1 May – 31 August) air temperatures were highly correlated between Earlton and Sudbury (r = 0.95, $p < 0.05$) during 1980–2002. This suggests that patterns in air temperature are regionally consistent and that the data employed in our analysis likely gave a good description of temporal patterns at our study lakes. However, mean values for summer sunshine were not as highly correlated between Kapuskasing and Sudbury (r = 0.63, $p < 0.05$). Mean summer wind speeds were not correlated between Earlton and Sudbury (r = 0.13, $p > 0.05$). Inaccuracies in our estimates for these weather variables for our study lakes, made from regional data, may therefore be a reason why they did not enter in our models. As well, ice-out dates for most years were estimated, not actually observed. More precise measurements of actual local conditions would undoubtedly lead to the development of more accurate models, and the collection of such data should be a priority in monitoring programs designed to assess the responses of lakes to climatic factors. Ideally, future monitoring in such remote locations would incorporate on-site instrumentation that provides continuous records of meteorology and lake thermal conditions.

Despite the data limitations, our simple regression models are a useful step toward better defining the temporal links between cold-water habitat, lake transparency, and weather conditions. The models developed for Whitepine Lake were successful in predicting the late-summer 10 °C depth in nearby Aurora Whitepine Lake, which is morphometrically similar and had a comparable (2.1–3.4 mg·L^{-1}) range in annual DOC concentrations. This indicates that these simple models may have widespread applicability to small, clear Boreal Shield lakes, although their generality will need to be further tested.

Much additional work, on additional lakes, will be needed to better quantify the relationships between weather, lake transparency, and thermal structure that we begin to explore here. Our results do, however, emphasize that in addition to direct impacts, important effects of climate change on lakes will be transmitted indirectly through lake and watershed processes that affect lake transparency. However, competing mechanisms may be involved.

In the thousands of North American lakes recovering from, or expected to recover from, acidification, the future

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relationships between acidification, DOC, and thermal structure will be complex. While pH and DOC concentration were positively correlated in Whitepine Lake, pH only explained 30% of the long-term variation in DOC. This suggests that over the relatively modest acidity change in Whitepine Lake (pH 5.7–6.3) during our study, other unmeasured factors, probably weather-related processes in the watershed, had the dominant effects on DOC. Hudson et al. (2003) also documented a pattern of DOC increases in lakes in south-central Ontario during a comparable time period (early 1980s to late 1990s), a temporal pattern that could be explained largely by variations in winter precipitation and solar radiation. In a much broader survey of Ontario lakes ($n = 662$) no trend in DOC concentration was the dominant response observed during the 1990s (Jeffries et al. 2003). Dramatic, direct, responses of DOC concentration to acidification and recovery from acidification have, however, been well documented, particularly when pH changes are large. In Swan Lake, near Sudbury, DOC concentration varied from 0.5 to 2.8 mg·L^{-1} as pH varied from 4.5 to 5.8 (Yan et al. 1996). In experimentally acidified Lake 302S in the Experimental Lakes Area, acidification from pH 6.0–6.7 to 4.5 caused the DOC concentration to decline from ~7.2 to 1.4–1.6 mg·L^{-1} (Schindler et al. 1996). A number of mechanisms may affect the response of lake DOC to acidification. These include DOC precipitation with increased concentrations of metals, particularly aluminum (Yan et al. 1996), and enhanced photochemical loss of DOC under acidic conditions (Gennings et al. 2001). Increased DOC and decreased clarity are usually observed as lakes recover from acidification (Dixit et al. 2001; Keller et al. 2003), although increased lake clarity during recovery has been observed in some ultraclear lakes (DOC < 0.5 mg·L^{-1}; Gunn et al. 2001).

In DOC concentration, Whitepine Lake is typical of many relatively clear waters on the Boreal Shield. The range of DOC concentrations during our study (2.1–3.3 mg·L^{-1}) falls in the most frequent interval (2–4 mg·L^{-1}, over 800 of 2581 lakes sampled) reported for Ontario lakes by Neary et al. (1990). In the USA, most of the acidic lakes dominated by inorganic acidity have comparably low DOC concentrations (median 2 mg·L^{-1}; National Acid Precipitation Assessment Program 1991). Thermal structure in such lakes is very sensitive to changes in transparency. Clark et al. (2004) have developed a model predicting 10 °C depth from DOC concentration and lake area. For a lake the size of Whitepine (67 ha), increasing the DOC concentration from 2 to 3 mg·L^{-1} is predicted by that model to decrease the 10 °C depth by 1.9 m, slightly less than the actual long-term decrease (2.6 m) observed in Whitepine Lake, which was also affected by an increasing trend in air temperature. Increasing the DOC concentration from 1 to 2 mg·L^{-1} is predicted to reduce the 10 °C depth by 5.7 m in a lake of that size.

The time scales of lake recovery in the large regions of North America affected by acidification are not yet predictable. Additional reductions in acid-forming emissions will need to be implemented to achieve recovery in many lakes (Jeffries et al. 2003). Thus, the decreases in lake clarity expected to result from decreases in lake acidity may interact, possibly for many decades, with changes in lake transparency and thermal structure related to a changing climate. It is apparent that major environmental stressors like climate change and acidification are linked through complex interactions (Schindler et al. 1996; Yan et al. 1996). These interactions must be much better understood if we are to make realistic predictions of the future effects of climate change in the large acid-affected regions of North America.

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Crustacean zooplankton in lakes and reservoirs of temperate and tropical regions: variation with trophic status

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Abstract: The influence of trophic status on the crustacean zooplankton community was investigated in lakes and reservoirs in temperate and subtropical–tropical regions. We tested if there is a consistent relationship between crustacean species richness, assemblages, and abundance and trophic indices such as total phosphorus and chlorophyll *a*. We also examined if these patterns differ between regions. Cumulative species richness and assemblages varied among regions. The greatest number of crustacean species was found in the temperate oligotrophic region with the largest number of lakes sampled. However, cumulative species richness was similar in temperate and subtropical–tropical regions when comparing subsets with a similar number of lakes and reservoirs. The relationships between species richness and latitude or trophic status were difficult to assess owing to imbalance among regions in number of lakes and reservoirs sampled and to biogeography and fish predation potential influences. Trophic status was associated with changes in abundance of all major crustacean zooplankton groups. Eutrophic ecosystems supported greater crustacean abundances at all latitudes. However, cladocerans and cyclopoids were more abundant in eutrophic lakes and reservoirs, whereas calanoids were more abundant in temperate oligotrophic lakes. Total phosphorus was found to be a better predictor of the biomass of major crustacean groups than chlorophyll *a* in all regions.

Résumé : L'influence du statut trophique sur la communauté de crustacés planctoniques a été évaluée dans des lacs et réservoirs des régions tempérée, subtropicale et tropicale. Nous avons analysé les relations existant entre la richesse en espèces, les assemblages et l'abondance des crustacés et les indices trophiques comme le phosphore total et la chlorophylle *a*. Nous avons testé si ces relations différaient entre les régions. La richesse cumulée en espèces et les assemblages de crustacés variaient entre les régions. Le plus grand nombre d'espèces a été trouvé dans les lacs oligotrophes de la région tempérée, les plus nombreux. Toutefois, la richesse en espèces dans chaque des régions était semblable lorsque l'on comparait des sous-ensembles ayant le même nombre de lacs ou de réservoirs. Les relations entre la richesse en espèces et la latitude ou le statut trophique étaient difficiles à évaluer à cause du déséquilibre dans le nombre de lacs et de réservoirs étudiés dans chaque région et de l'influence potentielle de la biogéographie et de la prédation par les poissons. L'abondance des groupes de crustacés variait avec le statut trophique. Les milieux eutrophes supportaient des populations de crustacés plus abondantes à toutes les latitudes. Toutefois, les cladocères et les copépodes cyclopoïdes étaient plus abondants dans les lacs et réservoirs eutrophes, tandis que les copépodes calanoïdes étaient plus nombreux dans les lacs oligotrophes tempérés. Dans toutes les régions, le phosphore total avait un meilleur pouvoir de prédiction de la biomasse de la majorité des groupes de crustacés que la chlorophylle *a*.

Introduction

The empirical relationships between aquatic communities and lake trophic status have been studied extensively. Consistent models relating phytoplankton biomass to nutrients or water transparency have been developed for both temperate and tropical lakes (Vollenweider 1976; Salas and Martino 1991). Despite some criticism and alternative proposals, the positive relationship between total phosphorus (TP) and phytoplankton chlorophyll *a* (Chl *a*) is one of the most robust global patterns in freshwater ecology. Recent efforts emphasize a more holistic approach, analyzing phytoplank-

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ton responses to lake trophy over large geographic scales or under different food web and morphometry conditions (Mazumder and Havens 1998).

Zooplankton have long been used as an indicator of lake trophy (Gannon and Stemberger 1978; Bays and Crisman 1982; Pejler 1983). Nonetheless, variable responses of zooplankton to trophic status are common (Ravera 1996), perhaps in part because zooplankton also respond to other environmental factors, such as lake water chemistry, shoreline disturbances, and watershed land use (Pinel-Alloul et al. 1990; Stemberger and Lazorchak 1994; Patoine et al. 2000) as well as levels of vertebrate and invertebrate predation (Hulot et al. 2000). Moreover, most studies dealing with zooplankton communities have focused on lakes with similar features located in restricted latitude ranges.

The main goal of this study is to test if there are consistent relationships between crustacean zooplankton community characteristics and trophic indices across a large geographic scale covering a variety of lakes and reservoirs in temperate and subtropical–tropical areas. The specific objectives are to (*i*) identify and compare the patterns of responses of species richness and abundance of major groups of crustacean zooplankton to increasing trophy in temperate and subtropical–tropical regions, (*ii*) test which indicator (e.g., TP and Chl *a*) of trophic status is the best predictor of the variability in crustacean zooplankton biomass, and (*iii*) test if the relationships between these trophic indicators and the biomass of crustacean zooplankton in lakes and reservoirs differ among regions.

Methods

Study regions

In carrying out a comparison of lakes in different geographic regions using preexisting survey data, it is an optimal strategy to establish if the lakes selected for study are unbiased representatives of the regions under consideration. In reality, however, the availability of similar data makes it difficult to reach this goal, especially when data from tropical regions are compared. In this study, we attempted to use the most representative data available in temperate and subtropical–tropical areas.

In the temperate zone, we used data from Canadian lakes covering a wide range of trophic states. The Coldwater region in northwestern Ontario (Fig. 1) is represented by five small, dimictic lakes (Table 1). They have mean depths less than 12 m and areas up to 57 ha and are oligotrophic, with low concentrations of Chl *a* (1–3 $\mu g \cdot L^{-1}$) and TP (4–8 $\mu g \cdot L^{-1}$). The Boreal Shield Sustainable Forest Management (SFM) region is represented by 38 headwater lakes in Quebec (Fig. 1; Table 1). Mean depth and area in these lakes range between 2 and 10 m and between 14 and 81 ha, respectively. They are oligomesotrophic with maximum Chl *a* and TP reaching 3 and 11 $\mu g \cdot L^{-1}$, respectively. The Terrestrial Riparian Organisms of Lakes and Streams (TROLS) region is represented by 12 small, shallow eutrophic lakes on the Alberta Boreal Plain (Fig. 1; Table 1), with higher levels of Chl *a* (6–33 $\mu g \cdot L^{-1}$) and TP (26–123 $\mu g \cdot L^{-1}$).

In subtropical Florida, data are available for five lakes (one lake including two basins) encompassing a wide range of trophic state (Fig. 1; Table 1). The lakes are located in the headwaters of the Kissimmee River, a major inflow to Lake Okeechobee. They have large surface areas (27–144 km^2) but very shallow depths (2–4 m maximum depth) and are eutrophic with Chl *a* concentrations being generally above 20 $\mu g \cdot L^{-1}$, except for two lakes below 6 $\mu g \cdot L^{-1}$. TP concentrations vary from 31 to 84 $\mu g \cdot L^{-1}$.

The tropical South America region was represented by reservoirs because natural lakes are scarce in that geographic region (Tundisi et al. 1981). Additionally, as most natural lakes in this area are very small and strongly influenced by flooding from their adjacent large river systems, they were not included in the comparison. The four Brazilian reservoirs differ widely in their trophic state. The Volta Grande reservoir (age 24 years, former land cover *cerrado* vegetation) is located in the lower Rio Grande, one of the rivers forming the Paraná River. It is an oligomesotrophic reservoir with an area of 205 km^2 and a volume of 2.3×10^9 m^3 (Table 1). Chl *a* concentrations were lower than 4 $\mu g \cdot L^{-1}$. Mean TP concentration was 14.3 $\mu g \cdot L^{-1}$ and ranged from 7 to 30 $\mu g \cdot L^{-1}$. Furnas Reservoir, located in the upper Rio Grande (Fig. 1), is one of the largest reservoirs of South America (age 41 years, former land cover *cerrado* vegetation). It has a flooded area of 1459 km^2, a volume of 2.3×10^{10} m^3, a maximum depth of 90 m, and a mean depth of 15.7 m. Trophic state varies within the reservoir, and TP concentrations are low (6–7 $\mu g \cdot L^{-1}$) in the central zone but reach higher values (35 $\mu g \cdot L^{-1}$) in the most eutrophic zones (Sapucaí River). Chl *a* ranges from 1–2 $\mu g \cdot L^{-1}$ in the central zone to 7–11 $\mu g \cdot L^{-1}$ in the eutrophic zones (Table 1). Pampulha and Ibiritié reservoirs are urban, shallow, and eutrophic and are located in the northern part of Belo Horizonte City, Brazil. The former land cover of both reservoirs consisted of transitional vegetation between *cerrado* and tropical rain forest. Both reservoirs have a small surface area (2.1–2.7 km^2) and mean depth (3.4–4.8 m) (Table 1). In Pampulha reservoir (age 64 years), Chl *a* concentrations range from 12 to 59 $\mu g \cdot L^{-1}$ and TP concentrations reach 269 $\mu g \cdot L^{-1}$. In Ibiritié reservoir (age 27 years), Chl *a* reaches a maximum of 50 $\mu g \cdot L^{-1}$ and TP ranges from 35 to 45 $\mu g \cdot L^{-1}$.

Further details about the limnological characteristics of the different ecosystems were previously presented in Carignan et al. (2000), Steedman (2000), and Prepas et al. (2001) for Canadian lakes, Havens et al. (2000*a*) for Florida lakes, and Pinto-Coelho (1998) for Brazilian reservoirs.

Sampling and analysis of zooplankton

The database included seasonal or annual means of crustacean zooplankton abundance (density and biomass). In the temperate region (Northern Hemisphere), samples were collected during the ice-free season (May–October). In Brazilian reservoirs, we took annual means excluding only data from periods of heavy rains. In Florida, the averages were based on bimonthly sampling over a 21-month period. The objective was to exclude periods with extreme conditions (ice-cover during winter in temperate regions and high flushing in the tropics) and thereby increase the possibility of detecting differences that could be attributed to trophic state.

In a large comparison of zooplankton from different regions, it is optimal if sampling methods do not vary in a manner that might bias the results and conclusions. In this study, most of the zooplankton were collected in the whole

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Fig. 1. Locations of the study lakes and reservoirs in North and South America.

water column with vertical net tows of small mesh size nets (53–150 μm) that retain most adult crustaceans (Table 2). The fixation and counting procedures were quite similar and the biomass estimations (milligrams dry weight per cubic metre) were done using length–biomass allometric equations.

The Coldwater lakes were sampled seven times during the ice-free season (May–October) from 1991 to 1995 summing 175 samples (Table 2). In the Boreal Quebec SFM lakes, sampling was carried out in 38 lakes in June, August, and September 1996 (114 samples). In the TROLS lakes, the data set includes 48 seasonal integrated samples obtained for each lake during the years 1995–1998. These composite year samples integrate 7–11 samples taken in each lake during the ice-free season. In Florida, sampling was conducted in the five lakes (at six collecting sites because the largest lake had two distinct segments) between April 1997 and January 1999 (98 samples). In Volta Grande reservoir, the sampling was done at a central site from November 1997 to January 1999 (14 composite samples). In Furnas reservoir, the data set includes 11 sites distributed in four regions in the reservoir along a trophic gradient from oligotrophic to eutrophic sites, and the sampling was done every 3 months between

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Table 1. Localization, morphometric characteristics, and trophic features of the selected ecosystems.

Region	Latitude	Longitude	No. of lakes (sites)	Area (km^2) (min.–max.)	Volume (m^3) (min.–max.)	Z_{max} (m) (min.–max.)	Z_m (m) (min.–max.)	TP ($\mu g \cdot L^{-1}$) (min.–max.)	Chl *a* ($\mu g \cdot L^{-1}$) (min.–max.)
Ontario Coldwater lakes	49°07′N	92°12′W	5	0.28–0.57	$1.7 \times 10^5 - 6.2 \times 10^5$	13.5–37	6.4–11.8	4–8	1–3
SFM lakes	48°50′N	75°00′W	38	0.14–0.81	$4.5 \times 10^6 - 8.5 \times 10^6$	5–34	2.0–10.0	7–11	2–3
TROLS lakes	55.1°–55.4°N	111.7°–113.8°W	12	0.14–1.57	$3.7 \times 10^6 - 7.4 \times 10^6$	2.1–9.4	0.6–4.7	26–123	6–33
Florida lakes	27–28°N	81°W	5 (6)	27–144	—	2–4	—	31–84	3–31
Volta Grande reservoir	19°–20°S	47°–48°W	1	205	2.3×10^9	19–22	8.2–11.2	7–30	1–4
Furnas reservoir	20°–21°S	45°–46°W	1 (11)	1459	2.3×10^{10}	85–90	10.7–15.7	6–35	1–11
Ibirité reservoir	19°S	43°W	1 (2)	2.7	1.0×10^8	11–13	3.4–3.7	35–45	8–50
Pampulha reservoir	19°S	43°W	1 (9)	2.1–2.4	$1.1 \times 10^7 - 1.2 \times 10^7$	16–17	4.8–5.2	25–269	12–59

Note: Data are ranges of seasonal means in epilimnion waters. Seasonal averages (May–October) were considered for temperate lakes. Annual averages were considered for Florida lakes and tropical reservoirs (excluding rainy period for the latter). Z_{max}, maximum depth; Z_m, mean depth; TP, total phosphorus; Chl *a*, chlorophyll *a*; SFM, Quebec Sustainable Forest Management; TROLS, Alberta Terrestrial Riparian Organisms of Lakes and Streams.

August 1999 and July 2000 (total of 48 samples). For Ibirité reservoir, the data set includes samples collected at two different sites every 2 weeks between August 1996 and January 1997 (12 samples). The data for Pampulha reservoir includes two sampling periods: October 1984 to November 1985 and February 1993 to November 1998. Samples were collected at three different stations in 1984–1985 and in a single station located in the central part of the reservoir during 6 years from 1993 to 1998 (total of 150 samples).

In the Coldwater, Quebec, and Alberta lakes, the zooplankton were sampled at the deepest point in each lake using a conical net or a cantilevering net with a 53-µm mesh size (Table 2). Vertical tows were made upwards through the water column from 1.0 m above the sediment to the surface. Filtered water volume varied from 80 to 1200 L. The biomass was estimated using length–weight equations (Malley et al. 1989). In Florida lakes, macrozooplankton were collected with two vertical tows of conical plankton net (30 cm in diameter, 150-µm mesh size). Tows were made from near the lake bottom to the water surface. Duplicate samples were pooled and preserved with 10% formaldehyde. In Volta Grande, Furnas, and Ibirité reservoirs, zooplankton were collected by means of vertical net hauls (65 cm in diameter, 90-µm mesh size) from 1.0 m from the lake bottom to the surface. In Pampulha reservoir, for the 1984–1985 period, zooplankton also were sampled using a conical 45-cm-diameter net with a 90-µm mesh size gauze, while a 5.13-L Schindler–Patalas trap with a 70-µm mesh size was used for the other periods. For subtropical and tropical regions, species biomass was estimated using length–weight equations provided by McCauley (1984).

Statistical analysis

To visualize and compare the effects of trophic status and latitude on crustacean zooplankton species assemblages, we used linear and nonlinear canonical correspondence analyses (CCAs) (ter Braak 1994; Legendre and Legendre 1998; Makarenkov and Legendre 2002). The annual maximum biomass of Cladocera, Cyclopoida, and Calanoida were considered as dependent variables. The independent variables were latitude, TP, and Chl *a*. All data were log transformed prior to analysis to better approximate normal distributions.

To assess and separate the possible effects of latitude and trophic status on each crustacean zooplankton group, we performed two-sample *t* tests. Following this, regressions between each zooplankton group and Chl *a* and TP were done. ANCOVA was used to evaluate and compare the regressions after first testing homogeneity of slopes. All analyses were performed using SYSTAT version 8.0 (SYSTAT Software Inc., Point Richmond, California).

Results

Species richness and crustacean community structure

Total cumulative species richness including all three groups of crustacean zooplankton (Cyclopoida, Calanoida, and Cladocera) was much higher in temperate oligotrophic lakes of Canada (region 1: 52 species) than in the other geographic regions or trophic categories (Table 3). Eutrophic lakes and reservoirs had lower cumulative species richness in both temperate (region 2: 26 species) and subtropical–

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Table 2. *Zooplankton sampling devices, net mesh sizes, sampling depths, study periods, and total sampling effort and samples in each lake region and reservoir.*

Region	Sampling device	Net mesh size (μm)	Sampling depth	Study period	Sampling effort per season and total samples
Ontario Coldwater lakes	Conical net	53	Total column	1991–1995: May–October	7 samplings per year, 5 lakes, 175 samples
SFM lakes	Cantilevering net	53	Total column	1996: June, August, September	3 samplings per year, 38 lakes, 114 samples
TROLS lakes	Conical net	53	Total column	1995–1998: May–October, composite samples	12 lakes, 4 years, 48 samples
Florida lakes	Conical net	150	Total column	1997–1999: 21-month survey	5 lakes, 6 sites, 98 samples
Volta Grande reservoir	Conical net	90	Total column	1997–1999: composite samples	1 site, 14 samples
Furnas reservoir	Conical net	90	Total column	1999–2000: 1-year survey	11 sites, 48 samples
Ibirité reservoir	Conical net	90	Total column	1996–1997: 6-month survey	2 sites, 12 samples
Pampulha reservoir	Conical net	90	Total column	1984–1985	3 sites
	Schindler–Patalas trap			1993–1998	1 site, 6 years, 150 samples

Note: SFM, Quebec Sustainable Forest Management; TROLS, Alberta Terrestrial Riparian Organisms of Lakes and Streams.

tropical regions (region 3: 21 species; region 5: 15 species). Oligotrophic tropical reservoirs also presented lower total species richness (region 4: 25 species).

Sixteen species of cyclopoids were found in the oligotrophic lakes in Canada (region 1 in Table 3), including the following genera: *Acanthocyclops*, *Cyclops*, *Diacyclops*, *Eucyclops*, *Mesocyclops*, *Orthocyclops*, *Paracyclops*, and *Tropocyclops*. In the eutrophic lakes in Canada (region 2), seven cyclopoid species were present. In Florida lakes (region 3), only three cyclopoid species were found (*Acanthocyclops vernalis*, *Mesocyclops edax*, and *Tropocyclops prasinus*); all of them also occurred in Canadian lakes. In the tropical reservoirs (regions 4 and 5), six cyclopoid species were recorded, at both eutrophic and oligotrophic sites. These species are endemic to tropical areas and were not found in the temperate or subtropical lakes (Table 3).

Calanoid species are more diversified in oligotrophic than in eutrophic systems, and they form a regional fauna, since no calanoid species co-occurred in the three regions studied (Canada, Florida, and Brazil). In the oligotrophic lakes in eastern Canada (region 1 in Table 3), nine calanoid species were recorded: *Aglaodiaptomus spatulocrenatus*, *Aglaodiaptomus* sp., *Epischura lacustris*, *Leptodiaptomus ashandi*, *Leptodiaptomus minutus*, *Leptodiaptomus sicilioides*, *Leptodiaptomus sicilis*, *Leptodiaptomus* sp., and *Skistodiaptomus oregonensis*. In the eutrophic lakes in western Canada (region 2), only three species of calanoids were present: *Acanthodiaptomus denticornis*, *E. lacustris*, and *S. oregonensis*. In Florida lakes, just two diaptomids were found: *Diaptomus dorsalis* and *Diaptomus floridanus*. Calanoids were relatively diverse in oligotrophic tropical reservoirs, where up to six different species (*Argyrodiaptomus furcatus*, *Notodiaptomus iheringi*, *Notodiaptomus* cf. *nordestinus*, *Notodiaptomus cearensis*, *Notodiaptomus henseni*, and *Scolodiaptomus corderoi*) were found, whereas only one species (*S. corderoi*) was recorded in the eutrophic tropical reservoirs.

One typical feature of cladoceran assemblages in the temperate region is higher species richness, especially for the genus *Daphnia* (Table 3). Fourteen species of *Daphnia* were found in the oligotrophic lakes in Canada (region 1). In the eutrophic lakes in Canada (region 2), the richness in *Daphnia* was lower but not less than eight different species were found. In contrast, 14 cladoceran species and only two *Daphnia* species (*Daphnia laevis* and *Daphnia gessneri*) were found in the Brazilian oligotrophic and eutrophic reservoirs (regions 4 and 5). The cladoceran community in Florida lakes (region 3) was relatively rich, with 16 species being reported, but it contained just two daphnids (*Daphnia ambigua* and *Daphnia lumholtzi*), one of which is an introduced exotic (Table 3). The predacious *Leptodora kindtii*, a common predator zooplankter in the Coldwater lakes, as well as the gelatinous *Holopedium gibberum* (Coldwater and SFM lakes) are missing in the Brazilian reservoirs. However, Florida lakes have some cladoceran species in common with Canadian lakes such as *H. gibberum*, *D. ambigua*, *Diaphanosoma brachyurum*, *Sida crystallina*, *Chydorus sphaericus*, and *Alona guttata*. Florida lakes also contain *L. kindtii* (K.E. Havens, personal observations), although none were contained in the samples evaluated here. Other species such as *Bosminopsis deitersi* and *Ceriodaphnia rigbaudi*, common in Florida lakes, are recorded in the tropical reservoirs as well. In Florida lakes, a number of noneuplanktonic cladoceran species like *Ilyocryptus spinifer*, *Macrothrix rosea*, or *A. guttata* also appear, probably associated with the high biomass of floating aquatic vegetation that develops during summer in these ecosystems.

The structure of zooplankton communities at different latitudes was further explored by CCA ordination of crustacean species assemblages (Fig. 2). The statistics associated with this analysis indicate that the variables describing trophic state (TP and Chl *a*) as well as latitude explained a high and significant proportion of the total variability in crustacean zooplankton community structure, expressed in terms of biomass of zooplankton groups. The mean coefficient of determination (R^2) was 0.70. The percentage of total variance explained by the first two axes was 69% and the sum of the two first canonical eigenvalues was 0.30 (first = 0.23, second = 0.07). Both linear and nonlinear models have statistical sig-

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Table 3. Crustacean species in the selected lakes and reservoirs of each region.

Species	Region 1	2	3	4	5
Cyclopoida					
Acanthocyclops carolinus		×			
Acanthocyclops vernalis	×	×	×		
Cyclops scutifer	×				
Diacyclops bicuspidatus thomasi	×	×			
Diacyclops nanus	×	×			
Dussicyclops navus	×				
Eucyclops serrulatus	×				
Eucyclops speratus	×				
Mesocyclops brasilianus					×
Mesocyclops edax	×	×	×		
Mesocyclops leuckarti	×	×			
Mesocyclops longisetus				×	×
Mesocyclops meridianus				×	×
Mesocyclops ogunnus				×	
Mesocyclops sp.	×				×
Metacyclops mendocinus				×	×
Orthocyclops modestus	×				
Paracyclops affinis	×				
Paracyclops poppei	×				
Thermocyclops decipiens				×	×
Thermocyclops minutus				×	
Tropocyclops prasinus	×	×	×		
Tropocyclops prasinus mexicanus	×				
Tropocyclops sp.	×				
Total	16	7	3	6	6
Calanoida					
Acanthodiaptomus denticornis		×			
Aglaodiaptomus sp.	×				
Aglaodiaptomus spatulocrenatus	×				
Argyrodiaptomus furcatus				×	
Epischura lacustris	×	×			
Diaptomus dorsalis			×		
Diaptomus floridanus			×		
Leptodiaptomus ashlandi	×				
Leptodiaptomus minutus	×				
Leptodiaptomus siciloides	×				
Leptodiaptomus sicilis	×				
Leptodiaptomus sp.	×				
Notodiaptomus iheringi				×	
Notodiaptomus cf. *nordestinus*				×	
Notodiaptomus cearensis				×	
Notodiaptomus henseni				×	
Scolodiaptomus corderoi				×	×
Skistodiaptomus oregonensis	×	×			
Total	9	3	2	6	1
Cladocera					
Acroperus harpae			×		
Alona guttata		×	×		
Alonella nana	×				
Alonella excisa		×			
Bosmina hagmanii				×	×
Bosmina longirostris	×			×	×

Table 3 (*concluded*).

Species	Region 1	2	3	4	5
Bosminopsis deitersi			×	×	
Camptocercus rectirostris			×		
Ceriodaphnia cornuta				×	×
Ceriodaphnia c. righaudi			×	×	×
Ceriodaphnia lacustris	×	×			
Ceriodaphnia megops		×			
Ceriodaphnia pulchella	×				
Ceriodaphnia sp.	×			×	
Chydorus sphaericus		×	×		
Chydorus piger	×	×			
Daphnia ambigua	×		×		
Daphnia catawba	×	×			
Daphnia dubia	×				
Daphnia galeata	×	×			
Daphnia gessneri				×	×
Daphnia laevis	×				×
Daphnia longiremis	×	×			
Daphnia longispina	×				
Daphnia lumholtzi			×		
Daphnia mendotae	×	×			
Daphnia middendorffiana	×				
Daphnia parvula	×	×			
Daphnia pulex	×	×			
Daphnia retrocurva	×				
Daphnia rosea	×	×			
Daphnia sp.	×	×			
Diaphanosoma birgei				×	×
Diaphanosoma brachyurum	×	×	×		
Diaphanosoma brevireme				×	
Diaphanosoma fluviatile				×	
Diaphanosoma spinulosum				×	
Diaphanosoma sp.	×	×			
Echinisca rosea			×		
Eubosmina longispina	×				
Eubosmina tubicen			×		
Holopedium gibberum	×		×		
Hyocryptus spinifer			×		
Leptodora kindtii	×				
Macrothrix rosea			×		
Moina micrura				×	×
Moina minuta				×	
Ophryoxus gracilis canadiensis	×				
Pleuroxus striatus			×		
Sida crystallina	×		×		
Total	27	16	16	13	8

Note: Region 1, oligotrophic lakes in Canada (Ontario Coldwater and Quebec Sustainable Forest Management lakes); region 2, eutrophic lakes in Canada (Alberta Terrestrial Riparian Organisms of Lakes and Streams lakes); region 3; Florida lakes; region 4, oligotrophic reservoirs in Brazil (Volta Grande and Furnas); region 5, eutrophic reservoirs in Brazil (Pampulha and Ibirité).

nificance ($P = 0.001$, 999 permutations), and the probability of a significant difference between the models was not significant ($P = 0.338$). Thus, we used the simpler (linear) com-

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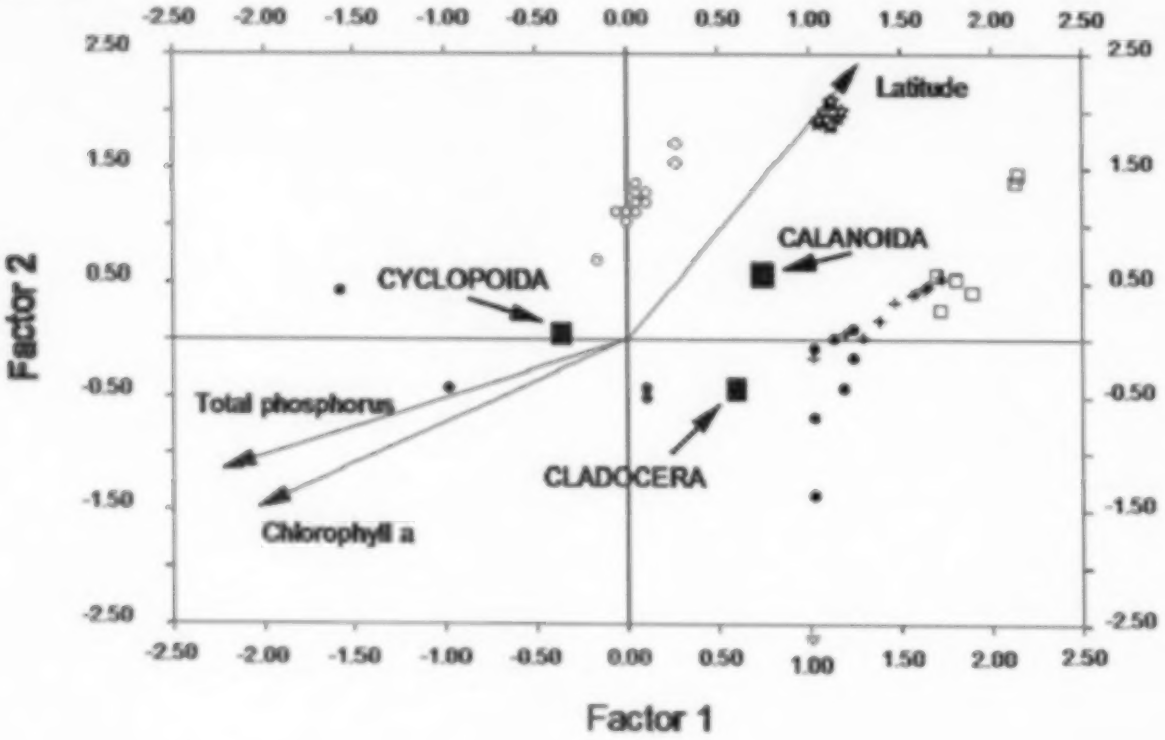
Fig. 2. Biplot diagram of canonical correspondence analysis (CCA). Zooplankton data used: maximum values of biomass of crustacean groups. Scaling of rows and columns: centering the biotic variables in relation to the site variables. Environmental variables: total phosphorus, chlorophyl *a*, latitude vectors (arrows). Biotic variables: Cyclopoida, Cladocera, and Calanoida (solid squares). Sites: region 1, temperate oligotrophic lakes (stars); region 2, temperate eutrophic lakes (open circles); region 3, subtropical Florida lakes (open squares); region 4, tropical oligotrophic reservoirs (crosses); region 5, tropical eutrophic reservoirs (solid circles). All data were log transformed.



bination of environmental and zooplankton variables in the CCA ordination (Fig. 2).

The two-dimension graph of CCA results (Fig. 2) clearly displays three main clusters of crustacean zooplankton associated with oligotrophic Coldwater–SFM lakes, eutrophic TROLS lakes, oligotrophic and eutrophic Brazilian reservoirs, and some of the Florida lakes. A fourth cluster contains two of the most oligotrophic Florida lakes (Fig. 2, upper right) and two sites from the Pampulha reservoir were spread in opposite direction (Fig. 2, lower left). The scaling procedure centered the scores (coordinates) of the biomass of the three crustacean groups in relation to the site scores. Cyclopoids and cladocerans were associated with the more eutrophic lakes and reservoirs. Cladocerans were positioned more closely to eutrophic tropical reservoirs, whereas cyclopoids appeared to be associated with the TROLS lakes and the hypertrophic Pampulha reservoir. Calanoids occupied a distinct position, in association with more oligotrophic ecosystems in both latitudinal regions.

The vectors of environmental variables were positioned in different regions of the CCA ordination (Fig. 2). In the negative region of axes 1 and 2, the variables were associated with trophic indices (TP and Chl *a*). The latitude vector appeared in the positive region of axes 1 and 2. This suggests the existence of different effects of latitude and trophic state on crustacean zooplankton structure.

Crustacean density and biomass

The comparison between annual means and annual maximum values of crustacean biomass and density revealed clear differences between tropical and temperate areas. The highest values of mean density and biomass were always observed in small eutrophic tropical systems for the cladocerans and cyclopoids, but no difference was found for the calanoids (Table 4).

The range of variability in the data was considerably greater in the small eutrophic tropical systems. The cyclopoids exhibited a large range of variation in abundance (density and biomass) across the trophic state gradients in temperate and tropical regions (Table 4). In temperate systems, cyclopoid mean density ranged from 14.2 individuals·L^{-1} (biomass = 7.5 mg dry weight·m^{-3}) in the Coldwater lakes to 45.2 individuals·L^{-1} (biomass = 13.6 mg dry weight·m^{-3}) in the TROLS lakes. The abundance of cyclopoids was low in the Florida lakes as well as in the large oligotrophic tropical reservoirs (density = 0.7–2.6 individuals·L^{-1}, biomass = 1.2–2.0 mg dry weight·m^{-3}). In the small eutrophic tropical reservoirs, cyclopoid abundance was much higher (density = 88.2 individuals·L^{-1}, biomass = 79.1 mg dry weight·m^{-3}).

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Table 4. Mean and maximum abundance (density and biomass) of major crustacean zooplankton groups in the selected temperate and tropical ecosystems.

	Cyclopoida				Calanoida				Cladocera			
	Density (individuals·L^{-1})		Biomass (mg dry weight·m^{-3})		Density (individuals·L^{-1})		Biomass (mg dry weight·m^{-3})		Density (individuals·L^{-1})		Biomass (mg dry weight·m^{-3})	
Lake regions	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean	Max.
Temperate regions												
SFM lakes	19.2	60.1	10.1	30.3	12.2	88.8	9.6	26.9	1.4	28.0	22.7	375.5
Ontario Coldwater lakes	14.2	62.4	7.5	68.7	7.7	43.9	10.6	59.9	3.8	19.0	2.8	18.3
TROLS lakes	45.2	266.2	13.6	116.3	18.5	98.8	10.3	56.9	10.5	63.2	14.8	133.8
Tropical regions												
Florida lakes	0.7	7.0	1.2	51.7	1.7	31.9	10.2	158.2	3.6	52.4	4.4	74.0
Large oligotrophic tropical reservoirs	2.6	9.8	2.0	14.4	1.7	7.5	2.1	11.9	7.3	66.1	8.2	92.4
Small eutrophic tropical reservoirs	88.2	2148.1	79.1	1829.7	9.1	175.4	7.6	102.7	34.8	286.5	169.9	4263.0

Note: In temperate regions, averages were based on ice-free months (May–October or June–September). In tropical regions, the averages refer to the annual cycle excluding the periods of heavy rains (months with more than 100 mm rainfall). SFM, Quebec Sustainable Forest Management; TROLS, Alberta Terrestrial Riparian Organisms of Lakes and Streams.

The mean density of calanoids in temperate regions ranged from 7.7 to 18.5 individuals·L^{-1} (Table 4). The biomass values of this group of crustaceans in Canadian lakes varied in a narrow range of values (9.6–10.6 mg dry weight·m^{-3}). In the tropical regions, calanoids always exhibited lower values of density (up to 9.1 individuals·L^{-1}) or biomass (up to 10.2 mg dry weight·m^{-3}).

As was the case for cyclopoids, cladoceran abundance increased along trophic gradients in temperate and tropical regions (Table 4). Their mean density increased from 1.4 individuals·L^{-1} in the oligotrophic Quebec lakes to 10.5 individuals·L^{-1} in the eutrophic TROLS lakes. It ranged from 7.3 individuals·L^{-1} in oligotrophic tropical reservoirs to 34.8 individuals·L^{-1} in eutrophic tropical reservoirs. Cladoceran mean biomass increased from 8.2 to 169.9 mg dry weight·m^{-3} along the trophic gradient in those reservoirs. In contrast, mean cladoceran biomass in the temperate region was lower in the more eutrophic TROLS lakes (14.8 mg dry weight·m^{-3}) than in oligotrophic Quebec lakes (22.7 mg dry weight·m^{-3}).

The CCA (Fig. 2) suggests the existence of some latitudinal and trophic influence on the structure of crustacean zooplankton. We tested if these effects can be isolated from each other and if they are significant (Table 5). The two-sample *t* test revealed that latitude has a significant relationship with mean density and biomass of calanoids, whereas trophy has no significant relationship with this zooplankton group. In contrast, the lake trophy gradient displayed significant differences in mean and maximum densities of cyclopoids, while latitude had no relationship with this group. The cladocerans were most strongly associated with these explicative variables, since both latitude and lake trophy are significantly related to variation in their mean and maximum density as well as trophy with mean biomass.

Table 5. Probability of accepting the null hypothesis (no difference between means) in the two-sample *t* test with crustacean zooplankton abundance (density and biomass) in response to the following treatments: latitude and trophy gradients.

	Density		Biomass	
	Latitude	Trophy	Latitude	Trophy
Cyclopoida				
Mean	0.925	0.029	0.223	0.066
Max.	0.229	0.035	0.173	0.112
Calanoida				
Mean	0.002	0.109	0.027	0.307
Max.	0.213	0.470	0.509	0.082
Cladocera				
Mean	0.015	0.000	0.054	0.026
Max.	0.006	0.000	0.116	0.102

Relationship of crustacean zooplankton to trophic state indicators

Since the CCA retained both Chl *a* and TP as explanatory variables, we investigated the relative power of these variables in explaining the responses of crustacean zooplankton across latitudinal gradients. A series of linear regressions using these independent variables and biomass of each major group of crustacean zooplankton as dependent variables was performed. Concentration of TP was the best predictor (Fig. 3) for total crustacean zooplankton, cyclopoids, and especially cladocerans. Previous results have demonstrated that calanoids prefer oligotrophic systems but their biomass values were not significantly correlated with TP or Chl *a* (Fig. 3). The assumption of parallel slopes could be accepted for the regression models of two major crustacean groups (Cyclopoida and Cladocera). This allowed us to perform the ANCOVA (Table 6) to test for differences in zooplankton – trophic state relationships across latitudinal regions. We found significant differences in the parameters of regression models for cyclopoids and cladocerans with TP (i.e., these regressions have significantly different slopes), suggesting different responses to TP in temperate and tropical regions. The analysis of TP–biomass relationships suggests that these

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Fig. 3. Scatterplots and linear regressions between mean biomass (log transformed) of total crustacean zooplankton, groups (Cladocera, Calanoida, and Cyclopoida), and trophic indices (log total phosphorus (TP), log chlorophyl *a* (Chl *a*)) in temperate and tropical ecosystems. Linear regressions parameters are presented in the legend when significant ($P \leq 0.05$).

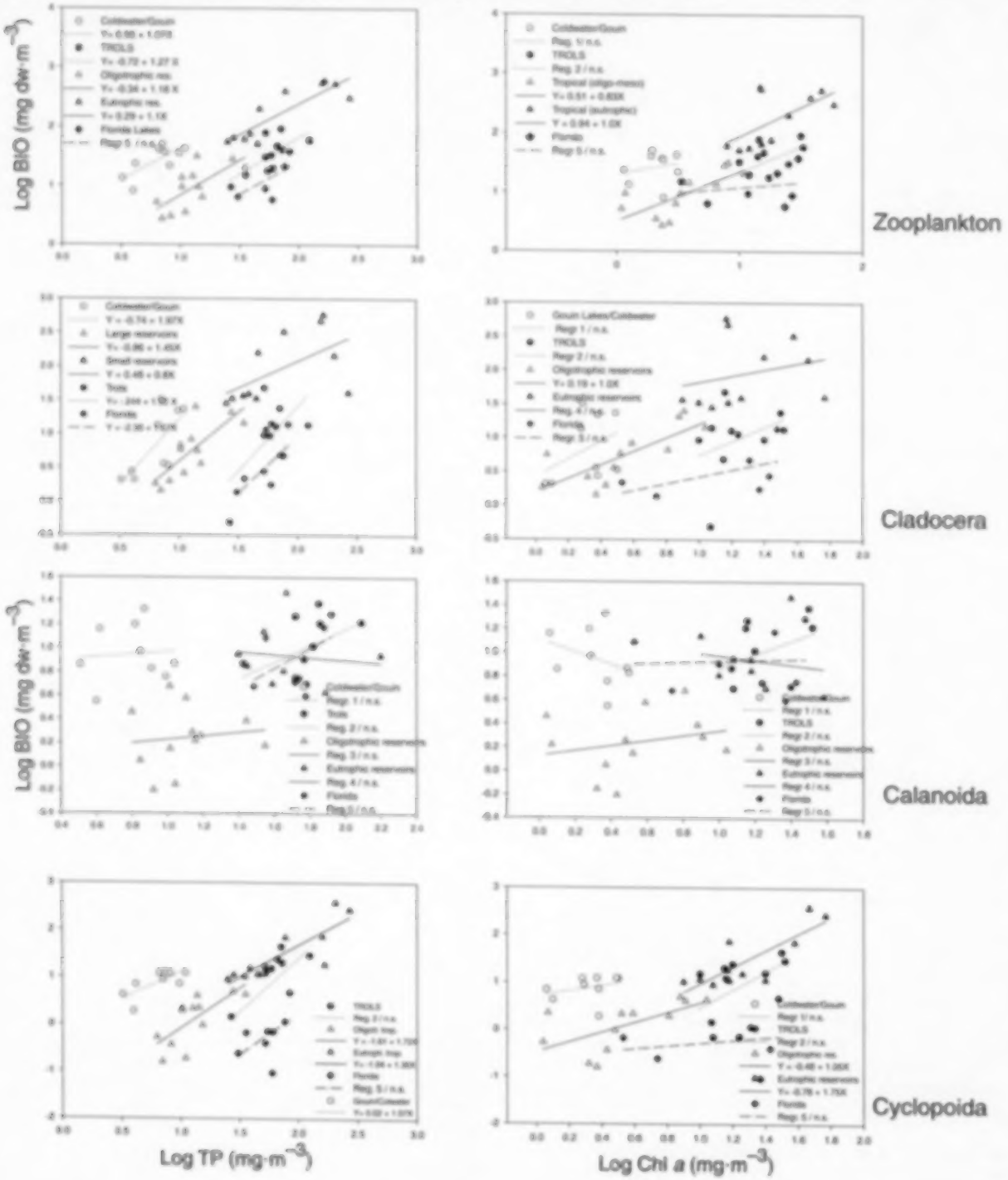

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Table 6. ANCOVA of biomass of zooplankton groups in response to eutrophication measured as total phosphorus (TP) and chlorophyl *a* (Chl *a*) and temperate and subtropical–tropical regions as treatments.

	Source	SS	df	MS	*F*	*P*
TP						
Cyclopoida	Treatment	17.931	4	4.483	23.228	<0.001
	Total phosphorus	6.364	1	6.364	32.975	<0.001
	Error	8.492	44	0.193		
Calanoida	Treatment	3.526	4	0.881	8.278	<0.001
	Total phosphorus	0.053	1	0.053	0.502	0.483
	Error	4.366	41	0.106		
Cladocera	Treatment	9.490	4	2.373	16.730	<0.001
	Total phosphorus	3.447	1	3.447	24.305	<0.001
	Error	6.240	44	0.142		
Chl *a*						
Cyclopoida	Treatment	15.786	4	3.946	16.220	<0.001
	Chl *a*	4.150	1	4.150	17.058	<0.001
	Error	10.706	44	0.243		
Calanoida	Treatment	3.458	4	0.865	8.048	<0.001
	Chl *a*	0.015	1	0.015	0.139	0.711
	Error	4.404	41	0.107		
Cladocera	Treatment	8.731	4	2.183	11.852	<0.001
	Chl *a*	1.584	1	1.584	8.601	0.005
	Error	8.103	44	0.184		

Note: Region 1, temperate oligotrophic (Quebec Sustainable Forest Management and Ontario Coldwater lakes); region 2, temperate eutrophic (Alberta Terrestrial Riparian Organisms of Lakes and Streams lakes); region 3, subtropical lakes (Florida); region 4, tropical large oligomesotrophic reservoirs; region 5, tropical hypereutrophic reservoirs. The homogeneity of slopes tests were positive for all groups but not for the total crustacean zooplankton.

responses are more intense in the tropical regions. A similar but weaker result was obtained using Chl *a* as the independent variable.

Discussion

Crustacean zooplankton taxonomic structure

In our study, we have used multiple-year sampling to estimate cumulative species richness at regional scale instead of single-year sampling, as suggested by Arnott et al. (1998). We found that oligotrophic lakes of the temperate region (SFM lakes in Quebec, Coldwater lakes in Ontario: region 1) host the richest zooplankton community, reaching a cumulative richness of 52 crustacean species. Other geographic regions have lower cumulative species richness, ranging from 15 to 26, the lowest species richness occurring in the eutrophic tropical reservoirs. The greatest number of crustacean species was found in the region with the largest number of lakes sampled. So despite having similar and large numbers of samples for both temperate (337 samples) and tropical regions (332 samples), one might argue that the observed spatial variation in species richness among temperate and subtropical–tropical regions is just a function of lake number and area (Dodson 1991; Arnott et al. 1998). To evaluate this possibility in our data sets, we estimated the average cumulative species richness that one would find if the number of lakes in the temperate oligotrophic regions would be equal to the number of lakes and reservoirs in the subtropical–tropical regions. We compared the average cumulative species richness for equal number of lakes in the temperate oligotrophic region (region 1) and lakes and reservoirs in the subtropical–tropical regions (regions 3–5). To conduct this assessment, we performed 1000 subsamples without replacement for each subset of increasing number of lakes (2–42) in temperate oligotrophic lakes (region 1: subsets of 2–42 lakes) and lakes and reservoirs in the subtropical–tropical regions, either when pooling Florida lakes with Brazilian reservoirs (regions 3–5: subsets of two to eight lakes and reservoirs) or when using separately the five subtropical Florida lakes (region 3: subsets of two to five lakes) and the Brazilian reservoirs (regions 4 and 5: subset of two and three reservoirs). Based on these subsamples, we calculated the average cumulative species richness for each subset of increasing number of lakes and reservoirs in each region (Fig. 4). We found that the average cumulative crustacean species richness in the temperate oligotrophic lakes (region 1) was lower than the values obtained in subtropical lakes and tropical reservoirs for subsets of lakes and reservoirs varying from two to eight (Fig. 4). However, high cumulative species richness in subtropical–tropical systems (regions 3–5) may be due to the great dissimilarity in species assemblages between Brazilian reservoirs and Florida lakes, the latter having more species in common with temperate regions than with tropical reservoirs. When separating Florida lakes (region 3) from Brazilian reservoirs (regions 4 and 5), the cumulative species richness for same subsets of lakes and reservoirs was slightly lower in subtropical lakes (around 20 species for five Florida lakes compared with 24 species in temperate oligotrophic lakes), and similar in the Brazilian reservoirs (21 species in average in subsets of three temperate oligotrophic lakes compared with 22 species in subsets of three tropical reservoirs). Thus, owing to unbalance among

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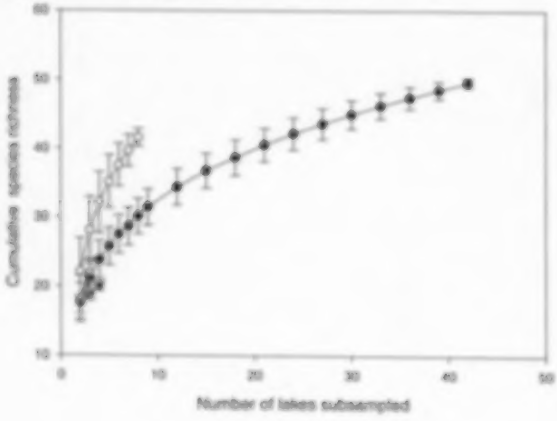
Fig. 4. Cumulative species richness (mean and error type) in subsets of increasing number of lakes and reservoirs in temperate oligotrophic lakes (region 1, solid circles) compared with subtropical–tropical regions: Brazilian reservoirs and Florida lakes pooled (regions 3–5, open circles), only Florida lakes (region 3, solid inverted triangles), and only Brazilian reservoirs (regions 4 and 5, open inverted triangles).



regions in the number of ecosystems studied, our study does not allow us to relate change in species richness between regions to latitude or lake trophy.

Finally, as deeper lakes often tend to be more oligotrophic, our results might also have been confounded by lake depth and size. To test this additional factor, we performed a simple linear regression between maximum and mean depth, area, and zooplankton species number on natural lakes, and we did not find any association between these variables.

Nevertheless, despite bias owing to difference in the number of lakes in the temperate and subtropical–tropical regions, the cumulative species richness estimated in each region was similar to other regional estimates. In temperate lakes in North America, Patalas (1975) found a total of 33 species in a survey of 14 large lakes. In northern Ontario, total crustacean species pool ranged from 21 to 40 species in lakes of similar morphometry and trophy to our temperate oligotrophic set of lakes (Keller and Pitblado 1989; Arnott et al. 1998). In Florida lakes, the cumulative species richness of 21 is quite similar to what has been previously reported for other lakes in the region (Crisman et al. 1995; Beaver and Havens 1996; Tugend and Allen 2000). Low diversity of crustaceans is a common feature of lakes in Brazil (Matsumura-Tundisi 1989; Bozelli 1992; Rocha et al. 1995). In a recent study, Sendacz and Junior (2003) reports only 16 species of mesozooplankton in a series of seven lakes of Paraná River in São Paulo State, Brazil, a similar value found in the selected reservoirs of the present study. One of the largest and deepest natural and oligotrophic lakes in eastern Brazil, Bispo Lake hosts only nine species of crustacean zooplankton (Matsumura-Tundisi 1997). Thus, we believe that our multiple-year survey and large sampling effort in both temperate and tropical regions give good estimates of crustacean species richness at the regional scale. In the Environmental Monitoring and Assessment Program – Surface Waters pilot survey of zooplankton richness in lakes of the northeastern Unites States, Stemberger et al. (1996) indicate that local species richness can be effectively extrapolated to the regional scale.

Among zooplankton groups, calanoid species richness is lower in eutrophic ecosystems than in oligotrophic ones in both temperate and tropical regions, as previously reported in temperate regions (Pace 1986). Calanoids seem to be sensitive to lake eutrophication and other lake watershed disturbances such as clearcut logging (Patoine et al. 2000). In the tropics, large calanoids like *E. lacustris* are absent, and calanoids often disappear from eutrophic lakes and reservoirs (Dussart et al. 1984; Matsumura-Tundisi 1986). Cyclopoid copepods also have lower diversity in the eutrophic systems in temperate lakes, whereas cladoceran species richness decreases in eutrophic systems within both tropical reservoirs and temperate lakes. In particular, the low diversity of *Daphnia* seems to be an outstanding feature of cladoceran assemblages in tropical systems, either oligotrophic or eutrophic. Matsumura-Tundisi (1984, 1989) found only 10 species of crustaceans and just one *Daphnia* species in the pristine Bispo (D. Helvécio) Lake and reported only three species of *Daphnia* for all fresh water systems in Brazil, where *Diaphanosoma* spp., *Moina* spp., and *Bosmina* spp. are the dominant species.

The low diversity of crustacean zooplankton found in the subtropical lakes of Florida and the tropical reservoirs of Brazil might also be a result of biogeography and fish predation. Peculiar geomorphology and geography of South America might also be important. Soto and Zuñiga (1991) observed that temperate lakes in Chile also have a much lower species diversity of crustacean zooplankton when compared with similar systems in North America. They explained these differences as a result of geographical isolation and species colonization history. The more diverse cladoceran fauna of Florida lakes also is probably a result of biogeography and peculiar ecological and morphometric conditions. These lakes occur relatively near to temperate areas of North America, in a very different biogeographic context, as compared with its counterpart systems in South America. These different geographic conditions could explain the differences between the available pool of species in tropical waters of Florida and South America. However, there are other studies suggesting that tropical waters usually have a lower diversity of zooplankton, especially for *Daphnia* species (Fernando et al. 1987). In a recent study, Aka et al. (2000) found no *Daphnia* species in a set of 49 reservoirs in the tropical Ivory Coast (Africa). In tropical lakes and reservoirs, fish predation is known to be a key factor structuring zooplankton communities, and the strong dominance of small copepods and cladocerans in tropical lakes (for instance, *D. dorsalis* in Florida lakes and *Moina* spp. in Brazilian reservoirs) and the disappearance of large cladocerans (*Daphnia* spp.) may be due to the high densities of zooplanktivorous fish (threadfin shad in Florida lakes and introduced African *Tilapia* in the Pampulha and Ibirite reservoirs) (Beaver and Havens 1996; Pinto-Coelho et al. 2000). The higher number of noneuplanktonic cladocerans in the Florida lakes may be associated with the presence of large stands of the submersed macrophyte *Hydrilla* that offer refuge against fish predation. The Florida lakes also have been colonized by exotic species, including *D. lumholtzi*, which is

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native to southeastern Asia, Australia, and eastern Africa (Havel and Hebert 1993; Havens et al. 2000*a*).

Crustacean density and biomass relationships

In our study, both the lowest and the highest densities (and biomass) of crustacean zooplankton were consistently found in the tropics, i.e., this region displayed the greatest interlake variability. This was particularly evident for the cyclopoids and cladocerans. High densities of total zooplankton (over 10^3 individuals·L^{-1}) are common in tropical waters. Crustacean zooplankton, especially small cladocerans and cyclopoids, can often reach such densities in hypereutrophic lakes, reservoirs, and fertilized ponds of hatchery stations (Branco and Senna 1996). Even the small calanoid *Scolodiaptomus corderoi* can occur and even form large populations in the most eutrophic reservoirs of Brazil (Reid et al. 1988).

The higher abundance of cladocerans found in tropical eutrophic waters might be a result of several interacting factors. Cladocerans typically have shorter development times compared with copepods and thus are able to exploit unstable and changing environments such as tropical reservoirs suffering from eutrophication. These systems often experience blooms of cyanobacteria and floating macrophytes. The increase in macrophyte beds creates a variety of new habitats favorable for zooplankton. Basu et al. (2000) observed a ninefold increase in biomass of zooplankton within dense macrophyte beds in fluvial lakes of the St. Lawrence River. The existence of long-lasting blooms of algae, such as cyanobacteria or filamentous diatoms like *Aulacoseira*, also creates favorable conditions for some smaller cladocerans such as *Bosmina longirostris* or *Bosmina hagmanii* (Branco and Senna 1996) or *Ceriodaphnia* (Ferrão-Filho et al. 2000). As stated before, the presence of efficient plankton feeders in Florida lakes (Beaver and Havens 1996) and Brazilian reservoirs (Pinto-Coelho et al. 2000) may favor the dominance of small copepods and cladocerans.

Reponses of zooplankton to trophic gradients

Our study confirmed that more nutrient-enriched lakes or reservoirs support greater crustacean zooplankton density and biomass. This is valid for both temperate and tropical regions. The regression models relating biomass of total crustacean zooplankton to TP were significant for most regions (excepting only Florida lakes) with R^2 varying between 0.45 and 0.77. The predictive power of our large-scale regression models is similar to that of other studies (R^2 = 0.63–0.86) done either at large geographic scales including 44 lakes mostly from temperate regions of North America and Europe (Hanson and Peters 1984) or at small regional scales in 16 nutrient-poor lakes in southcentral Ontario (Yan 1986) and 12 mesoeutrophic lakes in southern Quebec (Pace 1986). Patalas (1972) also found a significant regression (R^2 = 0.92) between crustacean abundance and phosphorus loading in the Great Lakes.

Considering previous studies and our results, it seems that there is a trend for crustacean zooplankton biomass to covary strongly with TP (Hanson and Peters 1984; Pace 1984; Yan 1986) and less with Chl *a*. A possible initial explanation could be the higher seasonal variability found for Chl *a* when compared with TP (Hanson and Peters 1984). However, there is an alternative explanation supporting this trend in tropical regions. The better power of TP for explaining the variability of crustacean zooplankton was also recently confirmed for tropical regions, such as the Pampulha Reservoir (Pinto-Coelho 1998). In this system, there is an imbalance between algae production and the biomass of zooplankton in reservoirs, since the assimilation rate of these consumers is not covered by phytoplankton production alone (Araújo and Pinto-Coelho 1998). In this kind of situation, where the detrital food chain is important, Chl *a* from algae cells certainly is not the best variable to be considered. Detritus food chains have also been found to be a major resource for zooplankton in a wide range of temperate lakes, and thus, TP has a better chance to reflect the total edible material than Chl *a* (Yan 1986). Likewise, Havens et al. (2000*b*) documented that phytoplankton-based food chains have low ecological transfer efficiency in a Florida lake and that much of the carbon and energy transfer to macrozooplankton must come from bacteria-based chains in the "microbial loop". Havens (2002) presented a simple conceptual model, based on zooplankton research in south Florida, indicating that while phytoplankton biomass is controlled by nutrients, zooplankton biomass is primarily controlled by productivity of bacteria. In systems of this type, it might be optimal to predict macrozooplankton biomass based on the combined biomass of phytoplankton and bacterioplankton. However, if effective predictions can be made based on TP, this would be the preferred approach owing to the much lower cost and effort.

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Monte Carlo evaluation of a nonlinear regression estimator for aggregated length–weight data

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Abstract: Parameters of fish length–weight relationships ($W = aL^b$) are usually estimated by applying linear regression to log-transformed length and weight values, but measuring individual weights is time-consuming and expensive. Often, length and weight data are available as sets of length measurements and aggregated sample weights, and the aggregate average weight of a sample can be expressed as the average of the weights predicted for the individual fish lengths. This study evaluated the feasibility of applying nonlinear regression to aggregated length–weight data. Experiments with simulated random length–weight data demonstrated that the estimates of parameter b appear to be unbiased and the estimates of a are right-skewed and biased. Further, the estimates of $\ln(a)$ and b are almost perfectly correlated. The precision and accuracy of the estimates were greatly influenced by the number of aggregate samples but were relatively unaffected by the number of fish in each sample. An additional experiment showed that the residuals from the regression can be used to detect small changes in the length–weight parameters.

Résumé : Les paramètres de la relation longueur–masse des poissons ($W = aL^b$) s'estiment généralement par la régression linéaire des valeurs de longueur et de masse transformées en logarithmes; la mesure des masses individuelles requiert cependant beaucoup de temps et de coûts. Il arrive souvent que des données de longueur et de masse soient disponibles sous la forme de séries de mesures de longueurs et de masses d'échantillons combinés; la masse moyenne combinée d'un échantillon peut alors être exprimée comme la moyenne des masses prédites pour les différentes longueurs de poissons. Notre étude évalue s'il est possible d'appliquer une régression non linéaire à des données combinées de longueur–masse. Des expériences faites avec des données aléatoires simulées de longueur–masse montrent que les estimations du paramètre b ne semblent pas faussées, mais que les estimations de a sont faussées et montrent une asymétrie vers la droite. De plus, les estimations de $\ln(a)$ et de b sont en corrélation presque parfaite. La précision et la justesse des estimations sont fortement influencées par le nombre d'échantillons combinés, mais elles sont relativement peu affectées par le nombre de poissons dans chaque échantillon. Une expérience supplémentaire a montré que les résidus de la régression peuvent servir à détecter de faibles changements dans les paramètres de la relation longueur–masse.

[Traduit par la Rédaction]

Introduction

A common practice in fishery science is to measure the lengths (L) and weights (W) of a sample of fish from a population and summarize the relationship using the power function $W = aL^b$.

If the fish do not change shape or density as they grow (isometric growth), then parameter b will have the value 3 and parameter a will be proportional to the density. This mathematical relationship is often used to convert length measurements to expected weight measurements or vice versa. The relationship can also be utilized to assess the condition of individuals or populations of the fish species (Murphy and Willis 1996). The two parameters a and b are generally estimated by applying a regression method, either ordinary linear regression on log-transformed variables or nonlinear regression on nontransformed variables (Hayes et al. 1995). Linear regression on log-transformed variables is the more common approach because of its conceptual and computational simplicity (Pienaar and Thomson 1969).

The two regression methods make fundamentally different assumptions about the underlying error structure. Linear regression applied to log-transformed weight and length measurements assumes that the errors in the observed weights are lognormally distributed and multiplicative on the arithmetic scale:

$$(1) \qquad W = aL^b\varepsilon$$

with $\ln(\varepsilon) \sim \text{Normal}(0, \sigma^2)$. Consequently, the parameters become linear with a normally distributed additive error term after logarithmic transformation. In contrast, the nonlinear regression applied to untransformed weight and length measurements assumes that the errors are normally distributed and additive:

$$(2) \qquad W = aL^b + \varepsilon$$

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with $\varepsilon \sim N(0, \sigma^2)$. If data conform to eq. 1, then fish that are long have greater variability in weight than fish that are short, whereas for data that conform to eq. 2, weight variability is homogeneous across all lengths.

For many United States west coast groundfish species, individual length and weight measurements are not routinely collected. However, length and weight data are widely available in the form of aggregate weights by sex for samples of fish plus the individual lengths of the fish in the samples. These aggregate sample weights and individual sample lengths are collected as part of the market sampling program in Oregon, whose primary purpose is to provide information for stock assessments on the sex, length, and age compositions of the landings (Sampson et al. 1997). Prior to 1989, most samples were taken with fixed numbers of fish per sample (e.g., 50 or 100 fish per sample, sexes combined). Subsequently, samples were taken on a fixed weight basis (e.g., 25 or 50 lb per sample, sexes combined (1 lb = 0.454 kg)). Similar kinds of data, individual fish lengths with aggregate weights, are sometimes collected during research trawl surveys when catches are too large for complete enumeration (Wakabayashi et al. 1985).

Nonlinear regression can be applied to these aggregated data to estimate the parameters of the length–weight relationship (Cammen 1980). For an aggregated sample of fish, which we refer to simply as a basket of fish, the total weight of the sample (T) is just the sum of the individual fish weights and can be predicted from the individual fish lengths:

$$(3) \qquad \hat{T} = \sum_{i=1}^{n} \hat{W}_i = \sum_{i=1}^{n} (aL_i^b)$$

If the errors in the observed weights are lognormally distributed, as in eq. 1, the observed weight of the jth basket sample is

$$(4) \qquad T_j = \sum_{i=1}^{n_j} W_{ij} = \sum_{i=1}^{n_j} (aL_{ij}^b \varepsilon_{ij})$$

Because of the summation on the right-hand side of eq. 4, logarithmic transformation of the equation does not result in any simplification and linear regression cannot be used.

If data are available from $M > 2$ baskets of fish, we can apply nonlinear least squares to find values for parameters a and b that minimize the sum across all of the samples of the squared differences between the observed and predicted total fish weights. If the number of fish in each basket (n_j) varies among the baskets, then using the average fish weight as the dependent variable rather than the total weight will help mitigate for the extra variability associated with samples having larger numbers of fish. The residual sum of squares (RSS) to be minimized becomes

$$(5) \qquad \mathrm{RSS} = \sum_{j=1}^{M} \left(\overline{W}_j - \frac{1}{n_j} \sum_{i=1}^{n_j} aL_{ij}^b \right)^2$$

where $\overline{W}_j$ denotes the observed average weight of the fish in the jth basket.

Application of the nonlinear method to data consisting of aggregated total weights and individual lengths was proposed and illustrated with simulated data by Cammen (1980). However, the simulation in that study generated only 10 replicate data sets and sample sizes were limited to 20 baskets with 10 or fewer fish per basket. Further, the experiment did not explore the influence of the characteristics of the length data. Hence, the reliability of this nonlinear regression technique and the properties of the estimates have not been fully explored.

The objectives of the current study were to determine whether the nonlinear regression method based on eq. 5 can produce reasonably accurate estimates of the parameters of the length–weight relationship, to identify factors that influence the accuracy of the estimates, and to test whether the residuals from the nonlinear regression method could be used to detect temporal changes in the length–weight relationship. The motivation for the last objective was to explore whether the nonlinear regression method could be used to assess changes in fish condition. The market sample data in Oregon have been collected on a regular basis for several decades. If the underlying fish weight versus length relationship changed during this period, those changes might be evident in the residuals from fitting a single nonlinear regression model to the long-term data series.

Materials and methods

To evaluate the nonlinear regression method based on eq. 5, we used Monte Carlo simulation experiments (Rubinstein 1981) and generated large numbers of replicate random data sets with known characteristics. From the simulated data, we derived estimates of the parameters of the length–weight relationship and compared them with the true known parameter values.

Market sample data characteristics

To develop appropriate data simulations for the Monte Carlo experiments, we first assessed the general characteristics of some actual data sets. Sample data on fish basket weights by sex and associated individual fish lengths were available from the Oregon Department of Fish and Wildlife for several fish species covering the period 1972–2000. We examined data for yellowtail rockfish (*Sebastes flavidus*) and Dover sole (*Microstomus pacificus*), which are major commercial species with long-term and relatively complete sampling coverage. The two species have very different body shapes, fusiform versus depressiform. We analyzed the individual fish length and weight data that were available for some samples, separately by sex, and estimated parameters (a and b) by linear regression applied to $\log_e$ transformations of both variables. We estimated the variance of the error term (σ^2) by the mean square error (MSE).

To understand the characteristics of the grouped measurement data and identify potentially influential factors, we examined the number of basket samples taken annually and the numbers of fish per basket as well as the distributions of fish lengths.

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Table 1. Fixed parameter values and experimental factor values used in experiments 1–5.

Parameter or experimental factor		Experiment				
		1	2	3	4	5
a	Length–weight relationship parameter	0.01	0.01	0.01, 0.0101,..., 0.0104	0.01	0.01
b	Length–weight relationship parameter	3.0	3.0	3.0, 3.03,..., 3.12	3.0	2.5, 3.0, 3.5
σ	Standard deviation in weight given length	0.12	0.12	0.12	0.12	0.12, 0.18,..., 0.36
M	Number of basket samples	10, 90	30, 60, 120,..., 960	30	50	50
μ_L	Average fish length	42, 46	44	44	44	44
σ_L	Standard deviation in fish length	4, 5	4	4	4	5
n_j	Number of fish per basket sample	10, 50	25	25	50	50

Note: Each experiment had 500 random replicate data sets.

Generating the random data sets

To simulate the length distributions, we used truncated normal distributions:

$$(6)\qquad f(L) = \begin{cases} \dfrac{\phi(L;\mu_L,\sigma_L)}{1-2\Phi(-\tau\sigma_L;\mu_L,\sigma_L)} \\ \\ 0 \qquad \text{otherwise} \end{cases}$$

$$\text{for } \mu_L - \tau\sigma_L < L < \mu_L + \tau\sigma_L$$

where L denotes the fish length and $\phi[L;\mu_L,\sigma_L]$ and $\Phi[L;\mu_L,\sigma_L]$ are the probability density function and cumulative density function for a normal distribution with mean μ_L and standard deviation σ_L. We made the truncation at $\tau = 3$ to prevent the generation of negative lengths and to control the ending points of the length distribution so that the effects of average length versus range of length could be more easily distinguished. The range $\mu_L \pm 3\sigma_L$ covers 99.74% of the total area of the normal distribution.

For each treatment in the experimental designs (Table 1), we generated the data and estimated the parameters using the following algorithm. (*i*) Generate a random length value L from the truncated normal distribution with the specified average and standard deviation. (*ii*) Using the length L from step *i*, generate a random weight value $W = aL^b \exp(\varepsilon)$ with $\varepsilon \sim N(0,\sigma^2)$. (*iii*) Repeat steps *i* and *ii* as necessary to generate the specified number of lengths and weights for a basket of fish. (*iv*) Round the fish lengths to the nearest integer values and tabulate the distribution of lengths and the corresponding basket weight. (*v*) Repeat steps *i*–*iv* as necessary to generate a data set with the number of baskets specified for the treatment. (*vi*) Estimate values for a and b for each data set using eq. 5 in the SAS procedure NLIN (SAS Institute Inc. 1994) with the Gaussian–Newton iteration method applied to the total fish weight and length frequency data. (*vii*) Repeat steps *i*–*vi* to generate 500 replicate data sets and 500 estimates of a and b.

For any fixed length L, the data simulator, which is based on eq. 1, generates lognormally distributed weights having an expected value of

$$(7)\qquad E[W] = aL^b \exp(0.5\sigma^2)$$

where the exponential term adjusts for the transformation of the random errors (Hayes et al. 1995). When evaluating bias in estimates of parameter a, one could argue that the bias should be measured relative to

$$(8)\qquad a' = a \exp(0.5\sigma^2)$$

which includes the transformation adjustment. For most experiments considered in this study, the random weights were generated with lognormal error and $\sigma^2 = 0.0144$. Hence, the adjusted value for parameter a was $a' = 1.0072a$.

Experiment 1: analysis of influential factors

Based on our preliminary examination of the market sample data, we considered four variables to be potentially influential in the estimation of the length–weight parameters: (*i*) the number of basket samples (M), (*ii*) the number of fish per basket sample (n_j), (*iii*) the standard deviation of the fish lengths (σ_L), and (*iv*) the average of the fish lengths (μ_L). We had various reasons for thinking that these factors would be influential. Sample size always has a major influence on the accuracy of statistical estimates, with larger samples producing more precise estimates. With our grouped measurement data, there are two different types of sample size: the overall number of basket samples in a data set and the number of fish in a given basket. At one extreme, there must be at least three baskets of fish (three measurements of weight) to provide sufficient information to estimate the two parameters of the length–weight relationship. At the other extreme, with one fish per basket, the problem reverts to the standard length versus weight regression. Hence, both types of sample size must influence the estimation of the length–weight relationship. In standard linear regression, the variance of the independent variable directly affects the precision of the estimated slope parameter. Unless there is variation in the independent variable, the slope parameter cannot be estimated and the standard error of the slope estimate decreases as the values of the independent variable become more widely spread. An analogous process probably occurs in our nonlinear regression problem. Finally, if the variability in a fish's weight is proportional to the fish's length, as often occurs in length–weight relationships (Ricker 1979), then whether the fish are short or long affects the variability in the basket weight as well as the estimation accuracy.

Experiment 1 followed a full factorial design using these four factors, each at two levels, for a total of $16 = 2^4$ treatments (Table 1). For the low and high levels for each factor, we chose values that we thought would amplify the potential influence of the factors but remain within ranges that were realistic based on our examinations of actual market sample data. To assess the bias of the parameter estimates, we used one-sample t tests that compared the mean of the estimates

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from each treatment with the underlying true value of the parameters used in the data simulations. To gauge the importance of the factors on the accuracy of the estimates, we applied analysis of variance (ANOVA) models using as response variables the squared deviations of either a or b from the parameter values fixed in the simulations:

$$(9) \quad \text{Deviation}^2 = (\text{Estimated} - \text{True})^2$$

To improve the normality of the response variables (the squared deviations) and stabilize the residual variance, we transformed the response variables before applying the ANOVAs using the log transformation for the deviations of the a estimates and the double square root transformation for the deviations of the b estimates.

Experiment 2: basket sample size effects

Because fish samplings are conducted in a random manner, the samplers have no control over the sizes of the sampled fish or the number of fish of a given sex in a basket sample. However, samplers could increase the number of basket samples to improve the accuracy of estimates. To examine the large-sample properties of length–weight parameter estimates, we simulated random data sets based on six basket sample sizes (M = 30, 60, 120, 240, 480, and 960) (Table 1) generated by doubling the baseline sample size of 30 baskets while keeping other factors fixed (n_j = 25, μ_L = 44, and σ_L = 4). The estimation method, simulation procedures, and other parameter values (a = 0.01, b = 3, and σ^2 = 0.0144) were as in the first experiment. We tested the bias of the estimates for a and b with one-sample t tests that compared the mean of the estimates from each sample size with the underlying true values of the parameters used in data simulation and used the root mean square error (RMSE) to measure their accuracy:

$$(10) \quad \text{RMSE} = \sqrt{E[(\text{Estimate} - \text{True})^2]}$$

$$= \sqrt{\text{Variance} + \text{Bias}^2}$$

Experiment 3: detecting length–weight changes from the residuals

Changes in the length–weight relationship over time, corresponding to changes in the condition of the fish, should be reflected in the residuals from a model applied to the complete data series. When building a model with a time-varying parameter, for example, $W = a(t)L^b$, one usually would first examine residuals from the model with constant a to guide the choice of using a linear or quadratic or other nonlinear function for $a(t)$. However, variation in $a(t)$ may be swamped by the underlying random errors in the data and therefore go undetected. To evaluate how sensitive the residuals from the nonlinear regression are to changes in the length–weight relationship, we simulated data as in the second experiment (n_j = 25, μ_L = 44, and σ_L = 4) (Table 1), but there were 30 basket samples (M = 30) for each of five time steps and the length–weight relationship changed systematically at each time step. For one data set, we fixed parameter b at 3 and incremented parameter a by 0.0001 at each time step, starting from 0.01, corresponding to a change of about 1% with each time step. Changes in parameter a would arise with changes in fish density. In a second data set, we fixed parameter a at 0.01 and incremented b by 0.003 at each time step, starting from 3.0, equivalent to a change of about 0.1% with each time step. Changes in parameter b would arise with changes in fish shape.

With both data sets, we pooled the five steps of data and analyzed them with a single nonlinear regression, as if the data were from a single population. We then applied ANOVA to the studentized residuals from the model to evaluate whether there were significant differences among the five time steps. To test which mean values were significantly different, we tested all pairwise combinations with the Student–Newman–Keuls multiple range test, keeping the experimentwise type I error rate at α = 0.05.

Experiment 4: alternative estimators

When individual length and aggregate weight data are available, an alternative approach for estimating the weight versus length relationship is to apply the standard length–weight regression model to the set of paired average length and average weight values from each sample:

$$(11) \quad \overline{W_j} = a(\overline{L_j})^b$$

When $b > 1$, this formula is only an approximation to eq. 1 because $\overline{L^b} > (\overline{L})^b$. However, the formula is much easier to apply than the nonlinear approach based on eq. 5. To evaluate this alternative formula, we conducted a small experiment, similar to experiment 1, with 500 randomly generated length and weight data sets, each with a = 0.01, b = 3.0, σ^2 = 0.0144, M = 50, n_j = 50, μ_L = 44, and σ_L = 4 (Table 1). To these data, we applied (A) the nonlinear regression method based on eq. 5 with average weight data and individual lengths, (B) nonlinear regression based on eq. 11 with average weights and average lengths, and (C) linear regression with log-transformed values of the average weights and average lengths (i.e., log transformation of eq. 11).

Experiment 5: influence of b and σ^2

Experiments 1 and 2 examined the behavior of the nonlinear regression method based on eq. 5 for the case of isometric growth (b = 3) and a fixed level of variability in weight given length (σ = 0.12 and σ^2 = 0.0144). To explore whether the results from those experiments might apply more generally, we conducted an additional set of experiments, in one case with simulated random data sets based on three values for parameter b (2.5, 3.0, and 3.5) with σ^2 = 0.0144, and in the other case with b = 3.0 and five values for σ (0.12, 0.18, 0.24, 0.30, and 0.36). In both cases, the other parameters and factors were fixed (a = 0.01, M = 50, n_j = 50, μ_L = 44, and σ_L = 5) (Table 1) and the estimation method and simulation procedures were the same as in experiment 1. We tested the bias of the estimates for a and b with one-sample t tests that compared the mean of the estimates from each sample size with the underlying true values of the parameters used in the data simulations.

Application to real data

We applied the nonlinear regression method to market sample data, consisting of aggregated weights and individual length measurements, for female Dover sole landed in Ore-

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gon after first screening out data from basket samples having fewer than five fish and those producing extreme studentized residuals (with absolute value greater than 4). From the nonlinear regression, we calculated the average studentized residuals by year to explore for long-term changes in the condition of these fish and by month to explore for seasonal changes. We limited our explorations to the studentized residuals for the period 1982–2000, when basket samples were available for almost all months of every year. During 1979–1981, sampling in the winter months was sporadic and prior to 1979 was limited to the months of May–September. We used one-way ANOVAs to test for differences among year and month effects. Missing data precluded a two-way analysis.

Results

Market sample data characteristics

Although Dover sole (a flatfish) and yellowtail rockfish (a round fish) differ considerably in their morphology, the parameter estimates for their weight and length relationships were similar, with the b estimates being around 3 and the a estimates around 0.01 g·cm^{-3} (Table 2). The estimates for the variance of the error term ranged from 0.0096 to 0.0128.

The numbers of basket samples per year and the number of fish per basket showed similar patterns for both species and sexes (Table 3). The numbers of samples taken annually were generally lower prior to the early 1990s, but the numbers of fish per basket were greater in the early years. Prior to 1991, most samples were collected with fixed numbers of fish per basket, 100 fish (sexes combined) prior to 1988 and 50 fish during 1988–1990. For both species and sexes, the length distributions were similar in general form with the distributions being fairly close to normal but slightly right-skewed. Further, the averages and standard deviations of length changed over time with a decreasing trend in average length over the years and variation in the standard deviation of length but no clear trend.

Experiment 1: analysis of influential factors

The estimates of a were highly skewed toward higher values and were biased for all treatments, with one-sample t tests rejecting for all treatments the hypothesis that the estimates of a were 0.01 ($P < 0.0001$) (Table 4). One-sample t tests also rejected for all treatments the hypothesis that the estimates of a were unbiased relative to the adjusted value of parameter a, $a' = a \exp(0.5\sigma^2)$. The estimates of a showed distinctly less variability for the treatments with the larger basket sample size and these treatments also had greatly reduced skewness (Fig. 1). For all treatments, the median values of a were very close to the true value, implying that the a estimates may be median unbiased. The one-sample t tests comparing the estimates of b from each treatment with the true value (3.0) provided no statistical evidence of bias; however, the variability of the estimates differed among the treatments. Most noticeable was the much lower variability from the treatments with the larger basket sample size. Box and whiskers plots displayed no apparent skewness in the estimates of b. Interestingly, there was an almost perfectly linear relationship ($r = -0.999\,975$) between the estimates of b and the logarithm of the a estimates. One-sample t tests of the $\ln(a)$ estimates revealed that they were not statistically different from the natural logarithm of the true a value, 0.01.

Table 2. Length–weight regression coefficients for two representative species, yellowtail rockfish (*Sebastes flavidus*) and Dover sole (*Microstomus pacificus*).

Species	Sex	a	b	σ^2	df
Yellowtail rockfish	Male	0.0158	2.9988	0.0096	2651
	Female	0.0173	2.9743	0.0126	2253
Dover sole	Male	0.0077	3.0406	0.0128	3784
	Female	0.0062	3.1058	0.0126	6181

Note: The parameters (a, b, and σ^2) were estimated using the linear regression model applied to log-transformed values of individual weights (g) and lengths (cm). The estimates of a have not been adjusted for transformation bias. df, degrees of freedom.

Given that the individual weights were generated with lognormal errors, one might surmise that the estimates of a would also be lognormally distributed. Anderson–Darling tests of normality (Stephens 1974) applied to the estimates of $\ln(a)$ found no significant ($P > 0.05$) departures from normality for 13 of the treatments but significant ($P < 0.01$) departures from normality for treatment 1 and highly significant ($P < 0.001$) departures for treatments 2 and 6 (Table 4).

The ANOVAs of the transformed squared deviations for each estimate demonstrated that the basket sample size (M) was the factor having the greatest influence on the accuracy of the estimates for both a and b (Table 5). Although the ANOVA models for the transformed a and b deviations were highly significant, they had low explanatory power, with R^2 values of only 18.9% for the a deviations and 29.5% for the b deviations.

In the ANOVA of the transformed a deviations, three of the four main effects were highly significant ($P < 0.01$) and the main effect for n_j (fish per basket) was close to significant ($P = 0.054$) but none of the interactions were significant ($P > 0.10$). However, factors other than M had very small influence. The MSE for M was 52 times larger than the second most significant factor, σ_L (standard deviation in length), and the coefficient for M was 7.2 times larger than the coefficient for σ_L. In the ANOVA of the transformed b deviations, the main effects for M and σ_L were highly significant ($P < 0.01$) and the main effect for n_j was significant ($P < 0.05$) but no other terms were significant ($P > 0.10$). Although σ_L and n_j were statistically significant factors, their relative influence was very small compared with M. The MSE for M was 48 times larger than the MSE of the second most influential factor in the model, σ_L, and the coefficient for M was 6.9 times larger than the coefficient for σ_L.

Experiment 2: basket sample size effects

For each basket sample size treatment, we tested the estimates of a and b for bias using one-sample t tests. For all sample sizes, the estimates of a were skewed toward higher values and the one-sample t tests confirmed that they were biased across all sample sizes (Table 6; Fig. 2). One-sample t tests also confirmed for all sample sizes that the estimates of a were biased relative to the adjusted value of parameter

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Table 3. Market sample data characteristics for two representative species, yellowtail rockfish (*Sebastes flavidus*) and Dover sole (*Microstomus pacificus*).

	Average value				Fish length (cm)			
	Baskets per year		Fish per basket		Average		SD	
Period	Male	Female	Male	Female	Male	Female	Male	Female
Yellowtail rockfish								
1976–1980	8.0	8.0	51.8	40.0	44.5	47.2	3.10	3.87
1981–1985	16.0	16.0	53.1	46.8	42.1	43.7	3.23	4.17
1986–1990	31.4	31.2	31.3	29.2	42.7	45.7	2.87	3.75
1991–1995	53.6	53.6	18.5	18.3	42.1	44.8	2.88	3.85
1996–2000	53.2	54.2	21.6	19.8	40.5	42.9	2.92	3.64
Dover sole								
1972–1975	16.3	16.0	35.6	65.6	37.0	40.1	3.06	4.53
1976–1980	12.2	13.0	34.0	66.3	36.6	40.3	3.08	4.74
1981–1985	33.8	34.0	52.2	56.8	35.6	39.4	2.92	4.50
1986–1990	49.0	49.4	38.7	33.8	34.7	38.5	2.91	4.51
1991–1995	53.4	54.6	27.6	24.0	34.7	38.8	3.18	4.87
1996–2000	35.2	36.0	23.0	26.1	34.6	39.1	3.09	4.75

Table 4. Parameter estimates from experiment 1, analysis of influential factors.

		Parameter *a*			Parameter *b*			
Treatment	Label	Min.	Mean	Max.	Min.	Mean	Max.	$P > \|t\|$
1	blsf	0.00002	0.0564	2.8306	1.500	3.032	4.691	0.140
2	blsF	0.0002	0.0261	0.2656	2.127	3.024	4.053	0.204
3	blSf	0.0001	0.0422	3.5030	1.444	2.997	4.311	0.874
4	blSF	0.0002	0.0304	0.8334	1.827	3.006	4.087	0.751
5	bLsf	0.0001	0.0536	1.2848	1.733	2.997	4.289	0.882
6	bLsF	0.0002	0.0315	0.4736	1.998	2.996	3.999	0.851
7	bLSf	0.00002	0.0483	1.9036	1.634	2.977	4.637	0.249
8	bLSF	0.0002	0.0296	0.5656	1.956	2.987	3.991	0.435
9	Blsf	0.0015	0.0120	0.0728	2.475	2.994	3.516	0.400
10	BlsF	0.0013	0.0117	0.0659	2.500	2.997	3.539	0.605
11	BlSf	0.0024	0.0114	0.0558	2.544	2.995	3.379	0.333
12	BlSF	0.0027	0.0113	0.0365	2.657	2.996	3.347	0.419
13	BLsf	0.0017	0.0121	0.0596	2.537	3.000	3.469	0.964
14	BLsF	0.0016	0.0121	0.0654	2.513	3.002	3.485	0.796
15	BLSf	0.0015	0.0113	0.0422	2.628	3.000	3.496	0.952
16	BLSF	0.0014	0.0109	0.0404	2.639	3.010	3.519	0.084

Note: The underlying true values of the parameters were $a = 0.01$ and $b = 3.0$. The P values from t tests that $b = 3$ are reported for each treatment. The P values from t tests that $a = 0.01$ were less than 0.0001 for all treatments. The lowercase and uppercase letters in the treatment labels denote the low and high levels, respectively, of the four factors, with B for the number of basket samples, L for the average fish length, S for the standard deviation in fish length, and F for the number of fish per basket.

a, $a' = a\exp(0.5\sigma^2)$. As the sample size increased, the degree of skewness in the estimates of a decreased considerably and the precision improved. The actual mean values of the a estimates became fairly close to the underlying true value ($a = 0.01$) for the sample sizes greater than 240, but the estimates of a were still right-skewed and were significantly different from the true value ($P < 0.001$). However, the mean values of the b estimates were not statistically different from the prespecified value ($b = 3$), suggesting that the estimator for b is unbiased, even for the smallest sample size used in the experiment.

The accuracy of the estimates of a and b, measured inversely by the RMSE, improved rapidly as the sample size increased to about 240 but showed only minor improvements at larger sample sizes (Fig. 3).

Experiment 3: detecting length–weight changes from residuals

Given changes in the parameters of the underlying length–weight relationships, the studentized residuals from the nonlinear regression models clearly showed differences among the residuals at each time step (Fig. 4). The ANOVA test of the studentized residuals associated with the steps confirmed that there was at least one significant difference among the mean values (F test, $P < 0.001$). We made pairwise comparisons for the means of the studentized residuals to gauge the

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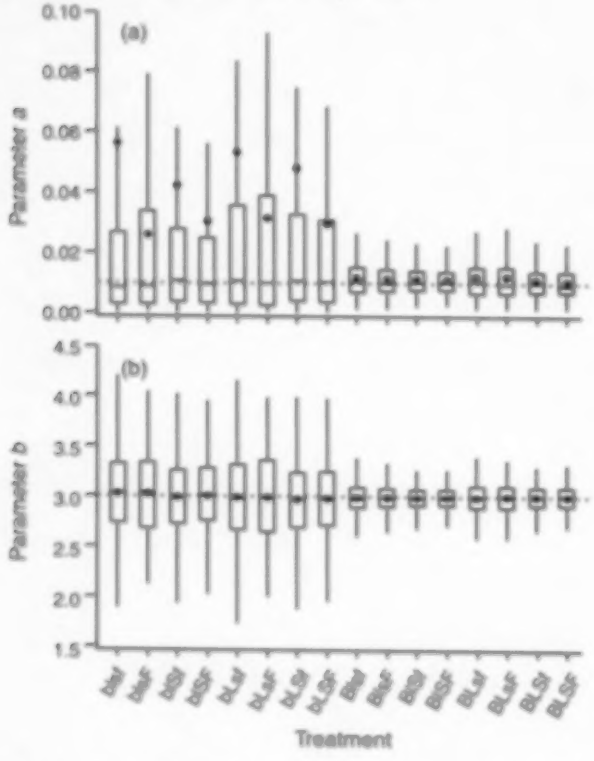
Fig. 1. Box and whisker plots of parameter estimates from experiment 1, analysis of influential factors. The circles indicate the mean of the estimates and the bars indicate the median values. The broken lines represent the true underlying values, $a = 0.01$ and $b = 3.0$. The lowercase and uppercase letters in the treatment labels denote the low and high levels, respectively, of the four factors, with B for the number of basket samples, L for the average fish length, S for the standard deviation in fish length, and F for the number of fish per basket.



significance of changes in residuals between pairs of time steps. For parameter *a*, the averages of the residuals from adjacent steps were not different from each other except between steps *ii* and *iii*, but the residuals from nonadjacent steps were significantly different from each other for all combinations. The results were similar for parameter *b*; the neighboring steps only differed for steps *iii* and *iv*, but all nonadjacent steps were significantly different from each other. The results imply that the residuals from a single nonlinear regression model fitted to the data series could be used to identify changes as small as 2% in parameter *a* and 0.2% in parameter *b*, given basket sample sizes of at least 30 for each of two time intervals.

Experiment 4: alternative estimators

The nonlinear regression method that we evaluated in experiments 1–3, method A with average weight data and individuals lengths, produced less biased and more accurate parameter estimates than the two methods that used data on average weights and average lengths. The estimates of *a* were positively biased for all three methods with method A, which used the individual lengths, showing the least bias, 27.5% versus 48.1% for method B, nonlinear regression, and 44.6% for method C, linear regression with log-transformed data. Method A also produced the most accurate *a* estimates, with relative RMSE equal to 100% versus 125% for method B and 121% for method C. The estimates of *b* were almost unbiased for all three methods with method A showing the least bias, 0.02% versus –0.98% for method B and –0.75%

Table 5. ANOVAs of the accuracy of the estimates of *a* and *b* from experiment 1.

Source	SS	df	*F*	*P* > *F*
Dependent variable[a]				
M	12 463.2	1	1810.89	<0.0001
μ_L	60.67	1	8.82	0.0030
σ_L	238.28	1	34.62	<0.0001
n_j	25.48	1	3.70	0.0544
$M \times \mu_L$	0.51	1	0.07	0.7855
$M \times \sigma_L$	12.21	1	1.77	0.1829
$M \times n_j$	0.00	1	0.00	1.0000
$\mu_L \times \sigma_L$	16.05	1	2.33	0.1268
$\mu_L \times n_j$	1.15	1	0.17	0.6827
$\sigma_L \times n_j$	0.16	1	0.02	0.8788
$M \times \mu_L \times \sigma_L$	6.52	1	0.95	0.3304
$M \times \mu_L \times n_j$	4.23	1	0.61	0.4331
$M \times \sigma_L \times n_j$	6.03	1	0.88	0.3493
$\mu_L \times \sigma_L \times n_j$	0.04	1	0.01	0.9392
$M \times \mu_L \times \sigma_L \times n_j$	0.84	1	0.12	0.7268
Error	54 948.6	7984		
Corrected total	67 784.0	7999		
Dependent variable[b]				
M	114.29	1	3256.53	<0.0001
μ_L	0.092	1	2.62	0.1057
σ_L	2.381	1	67.85	<0.0001
n_j	0.224	1	6.39	0.0115
$M \times \mu_L$	0.060	1	1.70	0.1926
$M \times \sigma_L$	0.019	1	0.53	0.4678
$M \times n_j$	0.059	1	1.68	0.1948
$\mu_L \times \sigma_L$	0.073	1	2.08	0.1493
$\mu_L \times n_j$	0.004	1	0.12	0.7294
$\sigma_L \times n_j$	0.003	1	0.07	0.7855
$M \times \mu_L \times \sigma_L$	0.026	1	0.74	0.3885
$M \times \mu_L \times n_j$	0.066	1	1.87	0.1716
$M \times \sigma_L \times n_j$	0.007	1	0.19	0.6622
$\mu_L \times \sigma_L \times n_j$	0.002	1	0.06	0.8068
$M \times \mu_L \times \sigma_L \times n_j$	0.000	1	0.00	1.0000
Error	280.20	7984		
Corrected total	397.50	7999		

Note: SS is the *adjusted* sums of squares and $P > F$ is the probability of exceeding that F by chance alone.

[a] $\ln[(\hat{a} - a)^2]$

[b] $\sqrt{(\hat{b} - b)^2}$

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Table 6. Parameter estimates from experiment 2, basket sample size effects.

	Parameter a				Parameter b		
Sample size	Min.	Mean	Max.	Skew(a)	Mean	CV(b) (%)	$P > \|t\|$
30	0.000 44	0.016 48	0.217 20	4.296	2.997	8.7	0.795
60	0.001 16	0.013 02	0.094 41	2.318	2.999	6.2	0.874
120	0.002 25	0.011 42	0.043 43	1.790	3.001	4.4	0.896
240	0.003 16	0.010 70	0.026 93	0.892	3.000	3.0	0.934
480	0.005 21	0.010 39	0.019 75	0.765	3.000	2.1	0.895
960	0.005 87	0.010 27	0.016 65	0.518	2.999	1.5	0.643

Note: The underlying true values of the parameters were $a = 0.01$ and $b = 3.0$. The P values from t tests that $b = 3$ are reported for each treatment. The P values from t tests that $a = 0.01$ were less than 0.0001 for sample sizes 30–240. $P = 0.0008$ for the 480 sample size and $P = 0.0012$ for the 960 sample size. Skew(a) is the skewness coefficient for the estimates of a. CV(b) is the coefficient of variation for the estimates of b.

Fig. 2. Box and whisker plots of parameter estimates from experiment 2, basket sample size effects. The circles indicate the mean of the estimates and the bars indicate the median values. The broken lines represent the true underlying values, $a = 0.01$ and $b = 3.0$.

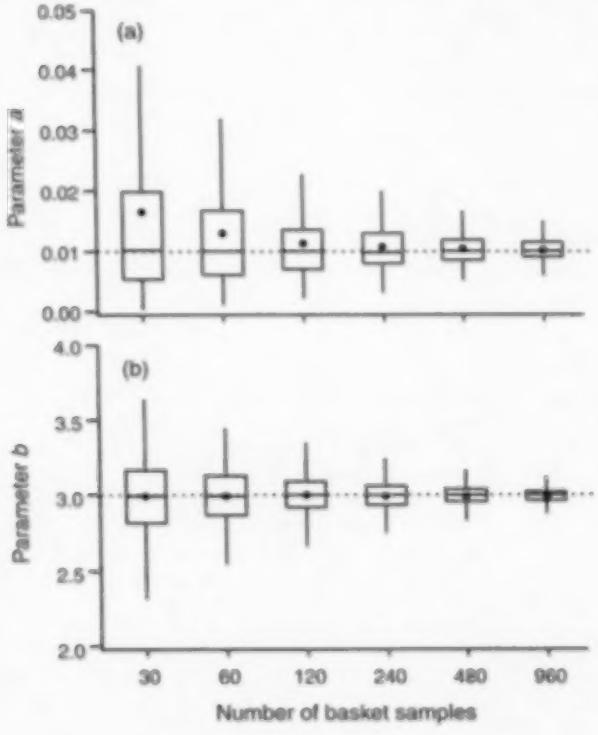
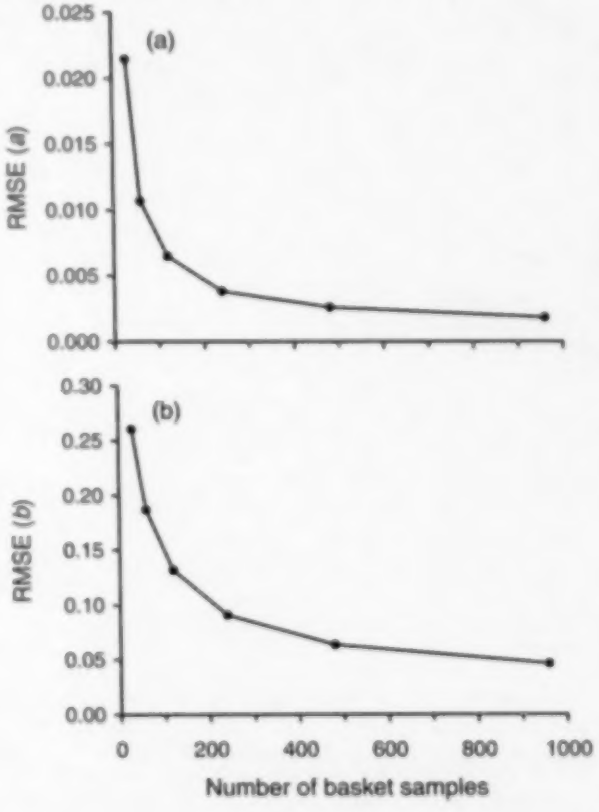


Fig. 3. Accuracy of the estimates of a and b from experiment 2, basket sample size effects.



for method C. Method A also produced the most accurate b estimates, with a relative RMSE of 6.1% versus 6.3% for methods B and C.

Experiment 5: influence of b and σ^2

For the data sets generated with three different treatment levels for the true value of b (2.5, 3.0, and 3.5), the estimates of a were highly skewed toward higher values and were biased for all three. One-sample t tests rejected the hypothesis that the average $\hat{a}$ value was 0.01 ($P < 0.0001$) and rejected the hypothesis that the estimates were unbiased relative to the adjusted value $a^* = a \exp(0.5\sigma^2)$. One-sample t tests comparing the average $\hat{b}$ from each treatment with the true values provided no statistical evidence of bias. Box and whiskers plots (Fig. 5) displayed no apparent skewness or differences in the distributions of $\ln(\hat{a})$ and $\hat{b}$ among the three treatments. Further, the estimates of b and the logarithm of the a estimates were almost perfectly correlated, with correlation coefficients ranging from –0.999 992 3 to –0.999 990 8.

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Fig. 4. Box and whisker plots of parameter estimates from experiment 3, detecting length–weight changes from residuals. (*a*) The true value for parameter *a* was changed by 0.0001 at each time step, starting from 0.01; (*b*) the true value for parameter *b* was changed by 0.003 at each time step, starting from 3.0. The circles indicate the mean of the estimates and the bars indicate the median values.

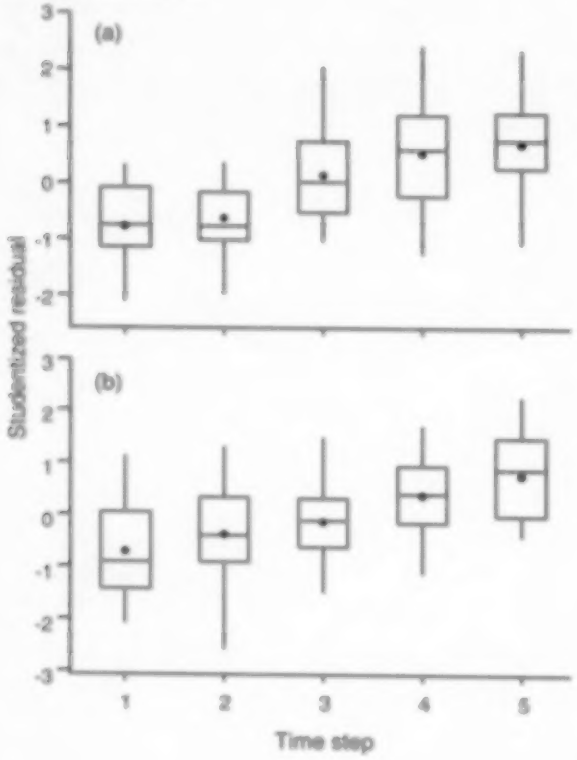
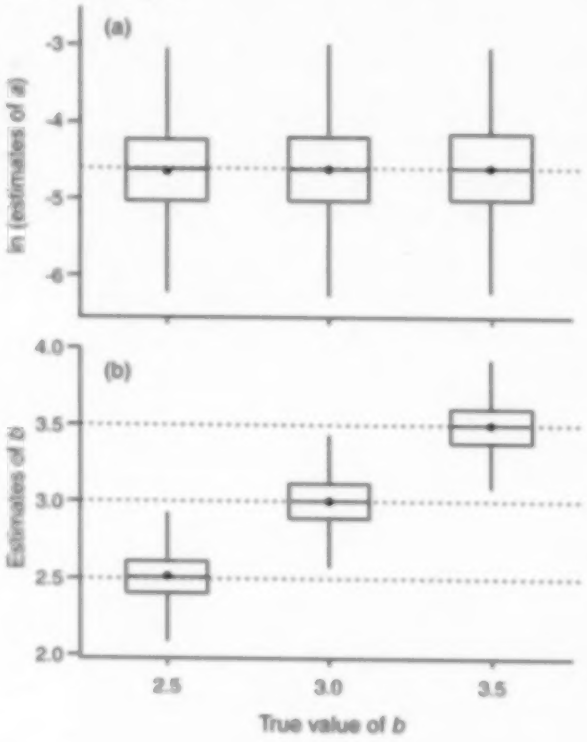


Fig. 5. Box and whisker plots of parameter estimates from experiment 5, the influence of *b*. The circles indicate the mean of the estimates and the bars indicate the median values. The broken lines represent the true underlying values, $\ln(a) = -4.605$ and $b = 2.5, 3.0, 3.5$.



For the data sets generated with five different treatment levels for the variability in weight given length (σ = 0.12, 0.18, 0.24, 0.30, and 0.36), the estimates of *a* again were biased and highly right-skewed. One-sample *t* tests rejected both the hypothesis that the average $\hat{a}$ value was 0.01 ($P < 0.0001$) and the hypothesis that average $\hat{a}$ value was $a' = a\exp(0.5\sigma^2)$. One-sample *t* tests comparing the average $\hat{b}$ from each treatment with the true value ($b = 3.0$) provided no statistical evidence of bias. Box and whiskers plots (Fig. 6) displayed no apparent skewness or differences in the distributions of the $\ln(\hat{a})$ and $\hat{b}$ values among the five treatments, although increasing variability in the estimates with increasing σ was clearly evident. Again, the values of $\hat{b}$ and $\ln(\hat{a})$ were almost perfectly correlated, with correlation coefficients ranging from –0.999 992 0 to –0.999 988.

Application to real data

During the period 1982–2000, aggregate weights and length composition data were available from 835 basket samples of female Dover sole. The parameter estimates derived from the nonlinear regression applied to this set of samples were $\hat{a}$ = 0.003 735 (SE = 0.000 512 6) and $\hat{b}$ = 3.2437 (SE = 0.0367), which are similar to the values that we estimated from the individual length and weight data that were available (Table 2) and also similar to the values reported for this species by Brodziak and Mikus (2000) ($\hat{a}$ = 0.004 414 9 (SE = 0.000 203 7) and $\hat{b}$ = 3.2254 (SE = 0.0128)). The average studentized weight residuals showed significant variation by year (one-way ANOVA, $F = 2.728$ with 18 and 816 df, $P < 0.001$). The average weight residuals were generally positive at either end of the series and strongly negative during 1990–1992 and 1994–1995 (Fig. 7*a*). The average weight residuals also showed significant variation by month (one-way ANOVA, $F = 7.98$ with 11 and 823 df, $P < 0.001$). The fish were unusually light during May and June and heavy during February and March (Fig. 7*b*). This pattern of seasonal change in condition is consistent with spawning cycle of this species, which is known to spawn during winter off Oregon (Hunter et al. 1992).

Discussion

The experiments in this study demonstrated that parameter *b* can be estimated with no apparent bias using the nonlinear regression method applied to group weight measurements and individual lengths. However, even with the largest samples, the estimates for parameter *a* were right-skewed and measurably biased, although the accuracy improved steadily as

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Fig. 6. Box and whisker plots of parameter estimates from experiment 5, the influence of σ^2. The circles indicate the mean of the estimates and the bars indicate the median values. The broken lines represent the true underlying values, $\ln(a) = -4.605$ and $b = 3$.

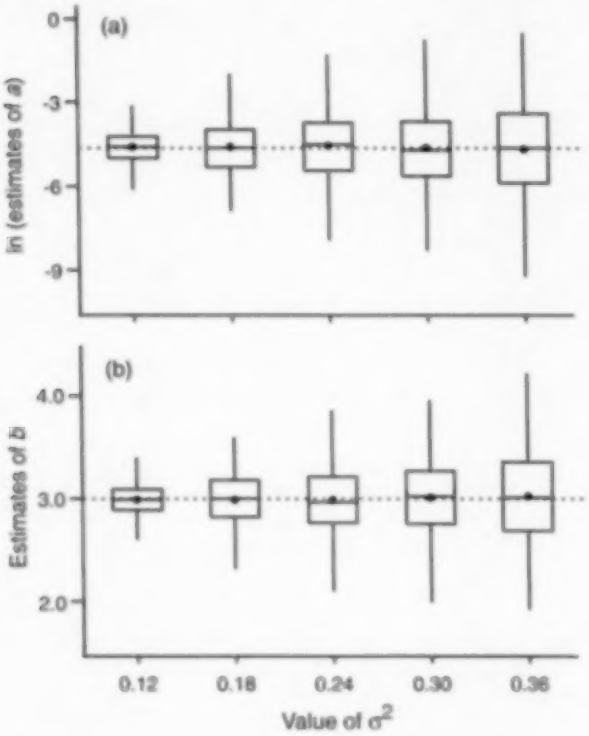
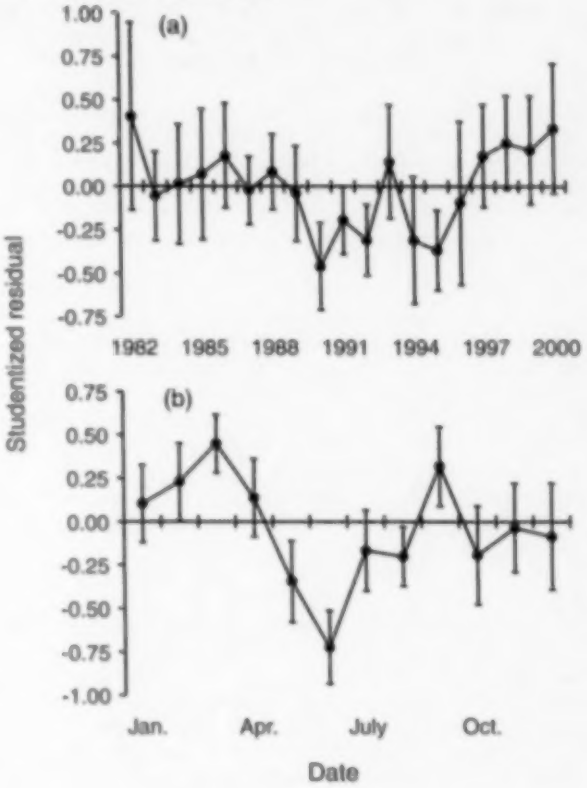


Fig. 7. Studentized average weight residuals (*a*) by year and (*b*) by month from application of the nonlinear regression method to aggregated weight and length data for female Dover sole (*Microstomus pacificus*) landed in Oregon. The error bars depict the mean values ± 2 SE.



sample size increased. The skew and bias of the *a* estimates may be caused by violation of the fundamental assumption in nonlinear regression of additive errors because we generated the data with multiplicative error. We used the multiplicative error structure because increasing weight variability with increasing length is a common feature of fish length–weight relationships (Ricker 1979).

Even in the linear model applied to log-transformed individual measurements of length and weight, the regression estimate of *a* is not free of bias. Transformation bias is introduced when the estimate of $\ln(a)$ is back-transformed by exponentiation to recover *a* because $\ln(a)$ is normally distributed but *a* is lognormally distributed. Although methods have been proposed to correct for this transformation bias (see Hayes et al. 1995 and references cited therein), they are not applicable to the nonlinear regression model that we used in this study. The bias in the estimates of *a* could be the result of inherent properties in the nonlinear model. Often, reparameterization or weighted regression can help to improve the performance of nonlinear regression models (Ratkowsky 1989), but we did not explore those possibilities in this study.

With the nonlinear regression applied to the aggregated data, the correlations between $\ln(\hat{a})$ and $\hat{b}$ seemed extraordinarily strong. To verify that this finding was unusual, we explored the correlation that one would obtain from the analogous linear regression model. If one applied linear regression to log-transformed individual length and weight data, the estimates of $\ln(a)$ and *b* would be linearly related with a theoretical correlation coefficient of (Appendix A)

$$(12) \qquad r[\ln(a), b] = \frac{-E[\ln(L)]}{\sqrt{V[\ln(L)] + E[\ln(L)]^2}}$$

where $E[]$ denotes the expected value and $V[]$ denotes the variance. For the length distributions that we explored in experiment 1, the theoretical correlation coefficients from linear regressions of individual ln(weight) on ln(length) data range from –0.999 74 to –0.999 49, whereas with the nonlinear regression applied to the aggregated data, we obtained correlation coefficients ranging from –0.999 995 to –0.999 956.

At least for the limited cases considered in this study, the nearly perfect linear relationships between the estimates of $\ln(a)$ and *b* obtained from the nonlinear regression method applied to aggregated data imply that the best approach for combining sets of *a* and *b* estimates from separate nonlinear regressions is to use the simple average of the estimates of *b* and the average of the log of the estimates of *a*. Directly averaging $\hat{a}$ values will produced a biased overall estimate.

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Because simulation experiments can only evaluate a limited range of treatments and parameter values, conclusions based on the restricted parameter space may not apply more generally. Although our experiments focused primarily on the case of isometric growth ($b = 3$) and relatively low variability in weight for a given length ($\sigma^2 = 0.0144$), the results from experiment 5, in which b and σ^2 differed from these baseline values, were consistent with the general results of the other experiments: the estimates of a were biased high, the estimates of b were unbiased, and the $\ln(\hat{a})$ and $\hat{b}$ values were very highly correlated. To verify these results for different sets of parameter values will require further experimentation. It seems unlikely that the results would depend on the value of parameter a, which is a simple multiplicative scalar, but the results could be influenced by the distribution of lengths.

Although it is simplest to apply linear regression to log-transformed lengths and weights measured from individual fish, it seems likely that data in the form of length frequencies and aggregated weights are available much more broadly. Fishery samplers routinely collect data on fish lengths for monitoring purposes and for use in formal stock assessments. When combining length frequency data collected from different fishing trips, the length measurements from larger landings should contribute more to the average length frequency than the data from smaller landings because there usually would be greater numbers of fish in the larger landings. To account for differing numbers of fish in the sampled landings, we can use the ratio of the weight of the landed catch over the weight of the sample to expand the sample length frequency data (Sampson 1994). Thus, when sampling for fish lengths, it is sensible to also record the corresponding total weight of the sampled fished. If, as we suspect, length frequency data are usually accompanied by the aggregate weights of the fish that were measured for length, then the nonlinear regression method that we describe and explore in this study provides a simple tool for estimating length and weight parameters from these data. Further, experiment 3 in this study illustrated that the average weight residuals from the nonlinear model are fairly sensitive to changes in the underlying length–weight relationship. Thus, the method can be used for assessing variations in growth condition over time or between populations.

Acknowledgements

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Appendix A

In the simple linear regression model:

$$Y = a + bX + \varepsilon \quad \text{with } \varepsilon \sim N(0, \sigma^2)$$

the estimates for parameters a an b must satisfy the relationship (Draper and Smith 1998)

$$\hat{a} = E[Y] - \hat{b}\,E[X]$$

Hence, with repeated sampling, the estimates of a and b will be distributed about the straight line having an a intercept equal to $(a + b)E[X]$ and slope equal to $-E[X]$. The variance of the a estimates is given by

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$$V[\hat{a}] = \frac{\sigma^2 \sum X^2}{n \sum (X - E[X])^2} = \frac{\sigma^2 E[X^2]}{nV[X]}$$

$$= \frac{\sigma^2 (V[X] + E[X]^2)}{nV[X]}$$

where n is the sample size. The variance of the b estimates is given by

$$V[\hat{b}] = \frac{\sigma^2}{\sum (X - E[X])^2} = \frac{\sigma^2}{nV[X]}$$

and the covariance of the parameter estimates is

$$\mathrm{Cov}[\hat{a}, \hat{b}] = \frac{-E[X]\sigma^2}{\sum (X - E[X])^2} = \frac{-E[X]\sigma^2}{nV[X]}$$

Hence, the correlation coefficient for the parameter estimates is

$$r[\hat{a}, \hat{b}] = \frac{\mathrm{Cov}[\hat{a}, \hat{b}]}{\sqrt{V[\hat{a}]V[\hat{b}]}} = \frac{-E[X]}{\sqrt{V[X] + E[X]^2}}$$

The correlation between the parameter estimates depends only on the mean and variance of the independent variable X.

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Selection equilibrium for hatchery and wild spawning fitness in integrated breeding programs

Daniel Goodman

Abstract: Some salmon hatchery programs intentionally integrate the wild and hatchery population by taking naturally spawned fish as some fraction of the broodstock and allowing hatchery progeny to constitute some fraction of the adults spawning in the wild. This circumvents some ecological concerns about the effects of hatchery fish on the "wild" population while still reaping some of the benefits of increased potential for harvest, but it increases some genetic concerns. Here, we model phenotypic evolution in the integrated population to investigate the effects on natural spawning fitness at the joint selection and demographic equilibrium. We find a potential, but not a certainty, depending on quantitative aspects of the management interacting with biological characteristics of the stock, for substantial erosion of natural spawning fitness, compared with the original wild population, including the possibility of runaway selection driving natural spawning fitness effectively to zero. The vulnerability to such evolutionary deterioration increases with the magnitude of the contribution of hatchery breeding to the total production and increases with harvest. The response of the selection equilibrium to increasing contribution of hatchery progeny to the broodstock can exhibit a catastrophic discontinuity.

Résumé : Certains programmes de pisciculture de saumons intègrent délibérément la population sauvage et la population de pisciculture en utilisant des poissons pondus en nature pour constituer une fraction du stock reproducteur et en permettant à des poissons nés en pisciculture de constituer une fraction des adultes qui se reproduisent en nature. Cette technique permet d'éviter certains problèmes écologiques concernant l'effet des poissons de pisciculture sur la population « sauvage », tout en maintenant certains des avantages du rendement accru à la capture; elle génère cependant des inquiétudes de nature génétique. Un modèle de l'évolution phénotypique de la population intégrée permet d'étudier les effets sur la fitness naturelle à la fraie dans des conditions conjointes d'équilibre de sélection et d'équilibre démographique. Selon les aspects quantitatifs de l'aménagement en interaction avec les caractéristiques biologiques du stock, il y a une possibilité, mais non une certitude, d'une érosion importante de la fitness naturelle à la fraie, par comparaison à la population sauvage d'origine, et même la possibilité d'une sélection galopante qui réduirait la fitness à la fraie effectivement à zéro. La vulnérabilité à une telle détérioration évolutive augmente en fonction de l'importance de la contribution de la reproduction en pisciculture à la production totale et elle augmente avec la récolte. La réaction de la sélection d'équilibre à une contribution croissante des rejetons élevés en pisciculture au stock reproducteur peut connaître une discontinuité catastrophique.

[Traduit par la Rédaction]

Introduction

Hatchery husbandry practices have advanced to the point where hatchery propagation of salmon can be an effective means to produce smolts for release to the wild, where they will rear, grow, and mature and return for harvest. While the smolt to adult return rates of hatchery salmon are often lower than those of the corresponding wild fish, the egg to smolt survival rates are generally enough higher in the hatchery, compared with the wild, to more than compensate. This results in a net numerical life cycle advantage for the hatchery stock in hatchery propagation, allowing a higher harvest rate. A salmon hatchery program that is successful from the standpoint of production for harvest may, nevertheless, be judged a liability because of harmful effects on wild salmon sharing the same natural habitats.

The negative ecological effects of hatchery production on a wild stock with which it interacts can include competition and predation (Waples 1999; Levin and Williams 2000; Levin et al. 2001). An unselective harvest managed for the higher productivity of the hatchery stock will overharvest the wild stock (Hilborn 1992).

Straying of hatchery adults into the wild spawning grounds raises the potential for harmful genetic effects as well. Hatchery propagation of a hatchery stock gives rise to domestication selection, which results in accumulation of adaptations to hatchery conditions, with attendant deterioration of performance under natural conditions during the corresponding portion of the life cycle. Further, the postrelease life history that evolves to mesh with the hatchery rearing may not prove adaptive when fish from the hatchery stock spawn in the wild. There is also a potential for inbreeding depression and loss of genetic diversity if the effective population size of an isolated hatchery stock is too small. Introgression between a domesti-

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cated or inbred hatchery stock and a wild stock will depress the fitness of the wild stock.

Concerns about the harmful effects of hatchery salmon stocks on their wild counterparts have prompted consideration of integrated production programs, where there is no importation of hatchery broodstock from other basins, where adults of local natural spawning origin are included among the hatchery broodstock in each generation, and a substantial number of adults of hatchery spawning origin are allowed to migrate to the natural spawning grounds. If the hatchery-origin fish spawn successfully with the natural-origin fish in the natural spawning grounds, the hatchery- and naturally spawning populations will converge genetically. In the end, there will be just one integrated population, with individuals that are derived from both hatchery and natural spawning in the previous generation and where fish of hatchery or natural spawning origin may cross over to the respective alternative spawning environment in any generation.

The hope, in advocating integrated programs, is that the merging of the hatchery and wild stocks will reduce the degree of domestication and inbreeding, which is a reasonable expectation, since a totally distinct hatchery stock is unlikely to diverge under these conditions and the effective population size is that of the integrated aggregate. It is still meaningful, however, to compare the properties of the population before and after such a program is initiated. In particular, we might inquire into changes in natural spawning fitness by comparing the natural spawning fitness in the absence of a hatchery influence with the natural spawning fitness that obtains at the selection equilibrium when a defined integrated production program is in effect. The present analysis will address that question by means of a model of demographic dynamics and phenotypic selection in a managed integrated production program.

Several versions of integrated salmon hatchery programs have been proposed, and several are underway (e.g., Carmichael and Messmer 1995; Dauble and Watson 1997; Fast and Craig 1997). Variations have to do with whether the intervention is intended to be temporary or indefinite, with the intended management of harvest, and with intended quantitative limits on the fraction of the naturally spawned run that may be taken as hatchery broodstock, the fraction of the broodstock that may be progeny of hatchery spawning, and the fraction of the adults on the natural spawning ground that may be progeny of hatchery spawning. The programs are variously called supplementation, augmentation, or supportive breeding, but the definitions are not consistent, and these terms are sometimes applied also to more conventional hatchery production programs that are still thought to serve some conservation role.

The question of the effects of integrated production on natural spawning fitness has been articulated previously (Campton 1995; Currens and Busack 1995; reviewed in Bilby et al. 2003). The theoretical possibility of the phenomenon has been treated explicitly in a number of modelling analyses. These models include one-locus two-allele classical genetic models of essentially antagonistic selection in the respective hatchery and natural environments (Reisenbichler 1984; Byrne et al. 1992; Harada 1992), single-trait quantitative genetics models specifically for a trait that can be conditionally deleterious in the respective hatchery and natural environments (Adkison 1995; Hard 1995; Ford 2002), models that also consider mutation and drift (Lynch and O'Hely 2001), and a more complicated quantitative model assuming some degree of coadaptation of traits (Emlen 1991) in the respective environments. With the exception of the analysis by Lynch and O'Hely (2001), the more comprehensive of these analyses were carried out by simulation or numerical solution for selected parameter values. These models show that evolution towards lower natural spawning fitness is indeed a theoretical possibility in integrated production programs.

In the present analysis, we focus exclusively on the effects of selection in the integrated population, ignoring the possible drift and inbreeding effects attributable to small population size. Further, we will assume that the set of geographic locations where broodstock is collected and where hatchery-phase products are released are chosen so that the hatchery phase does not merge multiple locally adapted stocks or inadvertently take broodstock selectively. Finally, we will not consider populations so near extinction that an immediate genetic or demographic rescue is needed.

The analysis will proceed in two main sections. First will be analysis of a model just of the demographic dynamics of a harvested integrated hatchery production system, operating under defined management protocols, primarily to obtain solutions for demographic equilibrium. The model will then be extended to consider the evolutionary equilibrium in such a system when the population encompasses a range of possible genotypes with different fitnesses in both the hatchery and natural spawning phases (Goodman 2004). The goal is to develop a comprehensive analysis that considers fitness as expressed in population dynamic terms in the context of a model that includes the effects of harvest and the controls on the breeding and release program.

In the interests of generality, the representation of particular genetic mechanisms will be bypassed in favor of a fitness set analysis that simply considers the spectrum of possible genetically determined phenotypes (Levins 1962; Schluter and Nychka 1994) and solves for the selection equilibrium as an evolutionarily stable state (Maynard Smith and Price 1973). This approach has the advantage of requiring fewer assumptions and fewer parameters. Basically, the evolutionary model is phenotypic, not genotypic, although it assumes heritability of phenotype. The assumptions of the model are unexceptional: (*i*) it postulates that evolution will maximize the overall effective fitness in the system, (*ii*) it diagnoses, via the demographic model, the respective contributions of hatchery spawning phase and natural spawning phase fitness to the overall fitness, and (*iii*) it assumes that there are inevitable trade-offs between achieved hatchery spawning fitness and natural spawning fitness. The analysis will dissect the relationship between the management controls of an integrated breeding protocol and the resulting relative weights that hatchery spawning fitness and natural spawning fitness will have in their respective contributions to overall fitness. These weights then determine the selection forces that will emphasize one fitness component at the expense of the other.

The generality of the approach does exact some cost in the range of properties about which the model can make pre-

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Table 1. Notation for elementary state description, dynamic parameters, and control variables.

State	
$N_{w,w,t}$	Number of naturally spawning fish in generation t that themselves grew from naturally spawned eggs
$N_{w,h,t}$	Number of naturally spawning fish in generation t that themselves grew from hatchery-spawned eggs
$N_{h,h,t}$	Number of fish used as hatchery broodstock in generation t that themselves grew from hatchery-spawned eggs
$N_{h,w,t}$	Number of fish used as hatchery broodstock in generation t that themselves grew from naturally spawned eggs
Dynamic	
R_w	Intrinsic replacement rate of a natural-spawning generation (female eggs per female) × (egg to smolt survival in nature) × (smolt to adult survival of naturally reared fish)
R_h	Intrinsic replacement rate of a hatchery-spawning generation (female eggs per female) × (egg to smolt survival in hatchery) × (smolt to adult survival, in nature, of hatchery-reared fish)
Control	
F_w	Fraction (after harvest) of the adult run of fish grown from naturally spawned eggs subsequently removed for broodstock
F_h	Fraction (after harvest) of the adult run of fish grown from hatchery-spawned eggs subsequently removed for broodstock
H_w	Fraction (before broodstock removal) of the adult run of fish grown from naturally spawned eggs that are taken in harvest
H_h	Fraction (before broodstock removal) of the adult run of fish grown from hatchery-spawned eggs that are taken in harvest

dictions. Because the model makes no assumptions about genetic mechanism, the results only can characterize the selection equilibrium. The analysis is silent about questions of the speed of the selection response, or the speed of the possible reverse evolution when supplementation is terminated.

Further, to promote generality, and hopefully to crystallize an efficient representation of the factors that control the fitness effects of selection in an integrated production system, the emphasis will be on analytical solutions, where possible, and where analytical solutions are not available, the results of systematic numerical explorations will be displayed to identify critical regions of the parameter space and to confirm robustness of the results.

Elementary notation

In the context of our question, we will use the term wild to designate a population that is not influenced by hatchery production, and natural spawning will refer to fish spawning in nature regardless of whether they themselves originated from hatchery or natural spawning. The notation for the elementary state description, dynamic parameters, and control variables is listed in Table 1.

The two inherent replacement rates R_w and R_h are measures of the biological performance of the stock in the respective circumstances of the natural life cycle and of the life cycle resulting from hatchery spawning and rearing to the smolt stage followed by the smolt to adult return stages in nature. Our question about selection in an integrated production system depressing the natural spawning fitness of the stock will be addressed in terms of the effects of this selection regime on R_w.

Density dependence

Over some large enough range of densities, the rates R_w and R_h must be density dependent, so, even assuming a constant environment and constant genotype, constancy of replacement rates, in the long-run, would only hold when the net dynamics, including harvest, just achieve replacement, thereby holding density constant. Supplementation is intended to increase the numbers of fish spawning in the wild, so it is to be expected that this will increase the intensity of density dependence effects, with a consequent depression of realized R_w compared with the presupplementation condition for as long as the supplementation is underway. If the density effect is so severe that there is a net loss (in total numbers, not just per capita) in the number of naturally spawned fish in the resulting adult run, then supplementation would be judged a failure on demographic grounds alone. This could occur, for example, with extreme overstocking of a population governed by a Ricker curve or for a population governed by a Beverton–Holt curve and already so near saturation that the natural spawning recruitment increase brought about by the supplementation is smaller than the amount of withdrawal of naturally spawned fish for broodstock. If the density effect is not so severe, the net effect could be an increase in the size of the naturally spawned run, notwithstanding the density-mediated depression in R_w. In this situation, the supplementation, at least temporarily, could be judged a demographic success. We note further that if the only force depressing R_w were the density dependence, the natural spawning replacement rate would return to its presupplementation level if the supplementation ceased and the densities returned to their presupplementation levels (unless overpopulation had caused some habitat or community composition damage that might take longer to recover).

The density-dependent depression of R_w, although merely an ecological rather than a genetic effect, may still have implications for the selection regime. Assuming that supplementation starts with a weak stock with, therefore, a low value of R_w, perhaps near 1, the density effect would likely reduce it further, possibly below replacement. If the hatchery replacement rate is high enough, it might still be possible to maintain a large number of spawners on the natu-

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ral spawning ground through the supplementation subsidy. But under these circumstances, the amount of supplementation would have to be large, and a large fraction of the adults on the natural spawning ground would themselves have been hatchery spawned rather than natural spawned. This would be contrary to common recommendations, reviewed in Bugert (1998) and Bilby et al. (2003), that the amount of supplementation and the fraction of hatchery spawned fish on the natural spawning grounds should both be limited in the hopes that such limitation will limit the consequent genetic effects.

Our concern in this paper is specifically with modelling the phenotypic fitness consequences if selection in the supplementation system causes genotypic change depressing the natural spawning replacement rate. This component of change in R_w would not be reversed immediately just by returning densities to their former levels. For clarity, we might envision the changes in R_w attendant upon supplementation to proceed in two steps, where the first is a density dependence response caused by the new population levels achieved by supplementation and the second is the inherently slower selection response that takes place over a period of generations while supplementation is under way, during which time the population in the natural environment remains at these elevated densities. To avoid the considerable complication of needing to model density dependence, we will take the initial density-dependent depression of R_w as given and confine our analysis to further changes brought about by selection. That is to say that the critical comparison will be between the preselection and postselection values of R_w at the elevated densities with supplementation underway rather than comparison with the presupplementation value of R_w (since that latter could involve a density effect as well as a selection effect). For this reason, we will refer to the preselection value of R_w as the baseline, where by "preselection" we mean prior to the effect of the new selection resulting from the supplementation regime.

Thus, we can envision scenarios where the control parameters F_w, F_a, H_w, and H_a are initially set by management to allow the population to grow or decline to the desired population size, and then the control parameters are adjusted, in relation to the R_w and R_a realized at that density, to achieve replacement at that density, and these adjustments continue to maintain the desired population size as R_w and R_a possibly change further as a result of selection. The modelling reported here will presume such adjustment, so that we need not model density dependence explicitly.

Demographic equilibrium

The life cycle dynamics determine the new state (the respective *N*s) from the previous state and the rate parameters, as diagrammed in fig. 2 of Goodman (2004). A difference equation representation of the four-element state description dynamics from one generation to the next is given by

$$N_{aa(t+1)} = (N_{aa(t)} + N_{aw(t)})R_a(1 - H_a)F_a \quad (1)$$

$$N_{aw(t+1)} = (N_{wa(t)} + N_{ww(t)})R_w(1 - H_w)F_w \quad (2)$$

$$N_{wa(t+1)} = (N_{aa(t)} + N_{aw(t)})R_a(1 - H_a)(1 - F_a) \quad (3)$$

$$N_{ww(t+1)} = (N_{wa(t)} + N_{ww(t)})R_w(1 - H_w)(1 - F_w) \quad (4)$$

We note that N_{aa} and N_{aw} only appear on the right-hand side of the equations as a sum, and N_{wa} and N_{ww} also only appear on the right as a sum. So, by defining $N_{a\cdot}$ as the sum of N_{aa} and N_{aw} and defining $N_{w\cdot}$ as the sum of N_{wa} and N_{ww}, we can reduce the dimensionality of the state description and the transition equations become

$$N_{a\cdot(t+1)} = N_{a\cdot(t)}R_a(1 - H_a)F_a + N_{w\cdot(t)}R_w(1 - H_w)F_w \quad (5)$$

$$N_{w\cdot(t+1)} = N_{a\cdot(t)}R_a(1 - H_a)(1 - F_a) + N_{w\cdot(t)}R_w(1 - H_w)(1 - F_w) \quad (6)$$

The transition eqs. 5 and 6 can be expressed as a matrix multiplication applied to a two-element state description vector:

$$\begin{pmatrix} N_{a\cdot} \\ N_{w\cdot} \end{pmatrix}_{t+1} = \begin{pmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{pmatrix}\begin{pmatrix} N_{a\cdot} \\ N_{w\cdot} \end{pmatrix}_t = \begin{pmatrix} R_a(1-H_a)F_a & R_w(1-H_w)F_w \\ R_a(1-H_a)(1-F_a) & R_w(1-H_w)(1-F_w) \end{pmatrix}\begin{pmatrix} N_{a\cdot} \\ N_{w\cdot} \end{pmatrix}_t \quad (7)$$

The asymptotic per generation growth rate of the population is given by the dominant eigenvalue of the transition matrix, which for the two-element system (eq. 7) is obtained from the larger of the two roots of the characteristic equation, namely:

$$\lambda_1 = \frac{(T_{11} + T_{22}) + \sqrt{(T_{11} + T_{22})^2 - 4(T_{11}T_{22} - T_{12}T_{21})}}{2} \quad (8)$$

Expressing the growth rate on a per generation basis allows comparison generally among systems with the same generation time. For the special case of exact replacement ($\lambda_1 = 1$), comparison is valid across systems with different generation times. The condition for exact replacement is

$$1 - (T_{11} + T_{22}) + T_{11}T_{22} - T_{12}T_{21} = 0 \quad (9)$$

which establishes the necessary relationship among the control parameters and the dynamic parameters for demographic equilibrium.

At replacement, we can solve for the dominant eigenvector of the transition matrix for the four-element state description (which has the same dominant eigenvalue as the matrix for the two-element system) to obtain the population composition at demographic equilibrium:

$$N^*_{aw} = N^*_{aa}\left(\frac{1}{R_a(1 - H_a)F_a} - 1\right) \quad (10)$$

$$N^*_{wa} = (N^*_{aa} + N^*_{aw})R_a(1 - H_a)(1 - F_a) \quad (11)$$

$$N^*_{ww} = \frac{N^*_{aw}}{R_w(1 - H_w)F_w} - N^*_{wa} \quad (12)$$

where the combination of F_w, F_a, H_w, and H_a must be such as to satisfy eq. 9 for that R_a and R_w and where the solution for the population composition solves only for ratios among N_{aa}, N_{aw}, N_{wa}, and N_{ww}, since eigenvectors are indeterminate to a scale factor.

The mean per capita harvest rate on the adult run at demographic equilibrium, therefore, is obtained as

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$$(13)\quad \bar{H}^{\circ} = \frac{H_n R_n (N^{\circ}_{nn} + N^{\circ}_{ns}) + H_s R_s (N^{\circ}_{sn} + N^{\circ}_{ss})}{R_n (N^{\circ}_{nn} + N^{\circ}_{ns}) + R_s (N^{\circ}_{sn} + N^{\circ}_{ss})}$$

where, again, F_n, F_s, H_n, and H_s must be such as to satisfy eq. 9 for that R_s and R_n and where substituting from eqs. 10, 11, and 12, the unknown scale factor cancels in the ratios of the N°_{ss}, N°_{sn}, N°_{nn}, and N°_{ns}.

Selection on hatchery and natural spawning replacement rates

Biological basis

The biological basis for expecting the genotype to undergo divergent selection in the evolution of R_s and R_n is that fish with the same genotype experience different adaptation problems in the two phases of the complex life cycle of the integrated population.

There is no credible disagreement about the reality of domestication selection under conditions of captive propagation in general (Arnold 1995), and more particularly in conventional hatchery programs with a hatchery stock (Reisenbichler and McIntyre 1977; Quinn et al. 1991; Utter et al. 1993), and specific frameworks have been proposed to measure it (Doyle 1983). There is a literature on the conservation potential for captive breeding that identifies breeding strategies to reduce the rate of some components of domestication evolution (Ballou et al. 1995) and rearing strategies to reduce the strength of some components of domestication selection (Ham and Pearsons 2001). Yet some degree of domestication selection is inevitable under artificial propagation, since evolutionary mechanisms are laws of nature that cannot be held in abeyance.

Domestication selection is not predominantly "artificial selection" in the sense of a deliberate direct culling to encourage retention of certain traits, as it is, more properly, natural selection in an artificial environment. As expected, passive domestication selection (as distinguished from deliberate artificial selection for traits associated with domestication and also as distinct from drift and inbreeding effects owing to "small" population size) improves hatchery performance (Wohlfarth 1986), and conversely, it is often observed that initial within-hatchery performance is not so good when a wild stock is taken into the hatchery to develop a hatchery stock (Doyle et al. 1995).

More of concern to our analysis are the life history changes that often attend domestication selection (Waples 1991; Mackey et al. 2001; Heath et al. 2003) and the possibility that these life history changes might be adaptations for the life cycle that begins with hatchery spawning and is completed with release and maturation in the wild but could be maladaptive for the life cycle that ensues when the same fish spawn naturally. There is evidence that selection on hatchery-reared fish after their release contributes to evolution of traits associated with domestication (Reisenbichler et al. 2004).

When domesticated fish from conventional hatchery programs are tested for performance under natural spawning conditions, they generally do poorly (reviewed in Independent Scientific Advisory Board (Bisson et al.) 2002). Some of these studies have specifically determined that some components of the effect are genetic (Johnsson et al. 1996; Currens et al. 1997; Reisenbichler and Rubin 1999). Some studies have shown that deliberate selection for faster growth in farmed salmon is associated with genetic change in other fitness-affecting traits (Fleming et al. 2002).

Natural selection between families is known to be strong in salmon (Geiger et al. 1997). The heritability of life history traits is high (Clarke et al. 1992; Clarke 1994; Gjedrem 2000). Rapid life history evolution has been observed often (Gharret and Thomasen 1987; Hendry et al. 2000), and some mixed life histories have been shown to be disadvantageous (Gharrett et al. 1999). This would argue that the life history changes observed associated with domestication are in fact adaptations to the hatchery-bred-released-to-outmigrate-and-mature life cycle and not just coincidental collateral damage from domestication. In a sense, then, the changed hatchery-based life history is as much a "local adaptation" as are the adaptations of wild stocks.

If we view a domesticated pure hatchery stock and an original wild stock as two distinct locally adapted stocks that coincidentally may overlap in space and time but that have evolved different adaptive solutions to different problems, we would expect introgression to produce offspring that are inferior in their performance in either of the environments of origin compared with the stock originating in that environment. This has been documented experimentally in steelhead (Reisenbichler and McIntyre 1977). Outbreeding depression in crosses between locally adapted wild stocks has also been documented in some nonsalmonid fish (Phillipp et al. 2002) and some other animals.

There is limited evidence from the field that the productivity of natural spawning salmon stocks is negatively related either to the frequency of hatchery-spawned fish on the natural spawning grounds (Chilcote 2003) or to the magnitude of hatchery smolt releases (Nickelson 2003), but these correlation studies are unclear about causal mechanism.

The foregoing review of evidence draws on experience with fish from conventional hatchery programs. Quantifying the life cycle performance of naturally spawning fish of hatchery origin in integrated hatchery programs and testing for genetic change have yet to be carried out systematically, most particularly in breeding programs with well-characterized control parameters (Waples et al. 2001). The limited available data suggest that the achieved multiplicative life cycle advantage in the hatchery phase in some integrated programs tends to be approximately twofold (Waples et al. 2001), which is lower than would generally be expected for a domesticated stock in a pure hatchery production program. There is some limited empirical evidence to suggest that an integrated breeding program may increase the aggregate population size (Waples et al. 2001), but there are documented cases where outplanting did not increase the size of the returning run (Nickelson et al. 1986). Some integrated programs with restrictive limitations on the rate of withdrawing adults of naturally spawning origin for broodstock have experienced difficulties in meeting these constraints (Bugert 1998).

Genotypic R_s and R_n as fitness components

We may represent combinations of values for hatchery spawning replacement rate R_s and natural spawning replace-

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ment rate R_w graphically as points in a Cartesian plane whose axes are R_a and R_w, as illustrated in fig. 1 of Goodman (2004). The (R_a, R_w) plane defines all of the mathematically possible genetically determined phenotype combinations for intrinsic replacement rates in the hatchery and natural spawning phases, respectively. The mean phenotype for any one genotype will correspond to one point in that plane. The overall fitness of that genotype, given a fixed management control regime specified by F_w, F_a, H_w and H_a, is simply the associated asymptotic rate of increase, λ_1, obtained from eq. 8. Isofitness contours in this plane, with a specified combination of controls, are obtained from solving for sets of (R_a, R_w) combinations that yield a particular fixed value of λ_1 with eq. 8.

At any location in the (R_a, R_w) plane, the slope of the isofitness contour at that location, as dR_w/dR_a, is found by implicit differentiation of eq. 8 with respect to R_a for constant λ_1. For the special isofitness contour corresponding to λ_1, the slope at any point on that contour is found by implicit differentiation of eq. 9 with respect to R_a and rearranging to

$$(14)\quad \left.\frac{dR_w}{dR_a}\right|_{F_w,F_a,H_w,H_a,\lambda_1=1} = \frac{R_w}{R_a}\left(\frac{T_{12}T_{21} - T_{11}(T_{22}-1)}{T_{22}(T_{11}-1) - T_{12}T_{21}}\right)$$

Possible set of replacement rate phenotypes

The (R_a, R_w) plane describes a phenotypic state space. If a population is fairly homogeneous phenotypically, it may be well characterized by its modal phenotype, which will correspond to a point in this state space. When a particular population undergoes selection in the traits associated with the two replacement rates, this will appear as movement, in this plane, of the point representing the population's phenotypic mode. Selection will favor any movement in the (R_a, R_w) plane in the direction of increasing fitness.

But not all of the mathematically possible combinations of R_a and R_w are biologically possible. Within the genetic and developmental repertoire of any population, there is a limited spectrum of possible phenotypes.

With respect to a defined set of traits, we define the spectrum of possible phenotypes in the population as the possible set. For the time scales that we are interested in here, on the order of at most a few tens of generations, the possible set will encompass the spectrum of phenotypes that can be constructed from the existing gene pool and the more frequent mutations, taking account of recombination, crossing over, and the multigenic nature of many quantitative traits. The responses to selection on this time scale are expected only to constitute microevolution, but this still may result in appreciable changes in traits of interest. For a given set of circumstances, evolution will favor the combination within the possible set that confers the highest fitness. For quantitative characters, it is to be expected that the possible set will consist of contiguous values of traits, so, geometrically, the possible set will be a coherent and enclosed portion of the state space, with a definable boundary.

For a set of genetically determined phenotype components that are all respectively positive fitness traits (as are R_a and R_w), the point in the possible set with the highest fitness value will necessarily be a geometric extremum. For any fixed value of R_a, fitness increases with increasing R_w, and for any fixed value of R_w, fitness increases with increasing R_a.

Therefore, the point with the highest fitness will lie somewhere on the boundary of the possible set. In the neighborhood of the point on the possible set with highest fitness, the slope of the boundary of the possible set will be determined by trade-offs in fitness characters arising from constraints, such as developmental, mechanical, and physiological limitations, and the life history options presented by the particular environment. Such trade-offs are commonly manifested in deliberate artificial selection programs where it is observed that continued directional selection for one trait eventually reaches a limit where there are strong correlations with other traits, and if those traits can be scaled as positive fitness characters, the correlations at the selection limit are negative.

The location on the boundary of the possible set where fitness is maximal will necessarily be a location where the slope of the boundary of the possible set is identical to the slope of the fitness isocontours. Otherwise, there would be a local gradient in fitness along the boundary of the possible set at that point, and therefore, there would be a direction of movement along the boundary of the possible set, which would increase fitness.

Interestingly, then, the slope of the border of the possible set of (R_a, R_w) combinations at a selection equilibrium (R_a^*, R_w^*) can be revealed by observing the phenotype at that equilibrium and the combination of management controls, F_w, F_a, H_w, and H_a, under which this equilibrium evolved, for that is sufficient to calculate from eq. 14 the slope of the λ_1 contour at that point, which in turn must be equal to the slope of the border of the possible set at that point.

For purposes of a local analysis, then, we may approximate the border of the possible set near the selection equilibrium as a straight line with the equation

$$(15)\quad R_w = \alpha R_a + b$$

where the necessarily negative slope α and the necessarily positive intercept b can be "back-calculated" from the static observation of the phenotype at the selection equilibrium and from knowledge of the controls under which it evolved. That is, α is obtained from the solution for dR_w/dR_a substituting the equilibrium values into eq. 14, and then b is solved from eq. 15.

Joint demographic and selection equilibrium

For sustainability, the equilibrium must confer a realized growth rate that is just at replacement. Thus, we are interested in a joint equilibrium, where the selection equilibrium (R_a^*, R_w^*) is located on the λ_1 isocontour.

A defined sustainable harvest policy

As a sufficiently productive population grows or declines under its own dynamics, the effects of density dependence on the realized replacement rates will bring it to a demographic equilibrium. In a managed system, of course, sustainability can be achieved over a range of population sizes through deliberate adjustment of the control parameters F_w, F_a, H_w, and H_a to satisfy eq. 9. We consider the situation of a managed population where the population size is al-

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ready at the desired level in relation to the carrying capacity of the available habitat, and we assume that management adjusts the various control parameters to keep the population at that size.

Assume that there is a policy to maintain a constant selectivity of the harvest, parameterized as s, a fraction between 0 and 1, such that

$$H_w = (1 - s)H_n \quad (16)$$

Then, with all other controls fixed, the base harvest rate (harvest on the hatchery spawned fish) that just achieves exact replacement is found by substituting eq. 16 into eq. 9, rearranging the equation as a polynomial in H_n, and then solving for the one positive root of the quadratic

$$H_n = \frac{-V + \sqrt{V^2 - 4Z(1 - s)}}{2Z(1 - s)} \quad (17)$$

where

$$Z = R_n R_w (F_n(1 - F_w) - F_w(1 - F_n)) \quad (18)$$

and

$$V = R_n F_n + R_w(1 - F_n)(1 - s) - (2 - s)Z \quad (19)$$

Evolution in response to management

Note that the possible set is a biological property of the stock in the context of its natural and hatchery environments. The possible set is not a property of the management controls. But the isofitness contours in the (R_n, R_w) plane do depend on the management controls.

Therefore, if the management regime changes through decisions to alter the harvest rates or the rates of removal of broodstock, the former selection equilibrium (R_n^*, R_w^*) will not necessarily continue to be the combination with the highest fitness under those conditions. Nevertheless, it will remain the case that the location of the former selection equilibrium (R_n^*, R_w^*) is somewhere on the border of the possible set, and the equation of the border of the possible set at that location is still given by the back-calculation from the former selection equilibrium and the controls under which it evolved.

Imagine a selection equilibrium at (R_n^*, R_w^*) that we now perturb by a slight change in the management regime. Consider a change in the direction of "more supplementation" where the amount of broodstock withdrawal is increased slightly through increasing F_n or F_w. Two things will happen. First, assuming obviously that there is a numerical life cycle advantage from hatchery production, the realized population growth rate will be increased, so the sustainability condition ($\lambda_1 = 1$) will be violated and the population will grow unless other controls are adjusted to compensate. Second, the intrinsic replacement rates R_n and R_w may begin to shift from their previous selection equilibrium, since the selection pressures will now have changed. What will the new selection equilibrium be after the shift in broodstock withdrawal rates as the harvest rate tracks the changes in realized productivity?

When the old equilibrium is perturbed by a change in broodstock withdrawal rate, we may imagine selection "testing" the values of R_n and R_w along the border of the possible set, where at each tested point, the harvests are adjusted by management for exact replacement. In one sense, all of the values of R_n and R_w tested by selection have the "same" fitness, since in the circumstances of harvest adjusted for replacement to satisfy eq. 9, all combinations confer $\lambda_1 = 1$. Nevertheless, not all of these combinations of R_n and R_w will persist as a selection equilibrium under this harvest policy.

At any location in the (R_n, R_w) space, with controls adjusted for replacement, mutant or low-frequency deviant genotypes can explore the neighborhood of (R_n, R_w) on the possible set border. If any such mutant or deviant can achieve a realized replacement rate greater than 1 under the current controls, that genotype will spread in the population. As this more productive phenotype spreads, and the population as a whole becomes more productive at a new (R_n, R_w), management will accordingly raise the harvests to enforce exact replacement. Thus, while the population's realized fitness will be held at a constant value, $\lambda_1 = 1$, by management control, particular genotypes may temporarily achieve higher fitness and spread in the population, and so the population's intrinsic net replacement characteristics (R_n, R_w) can evolve.

The only circumstance under which (R_n, R_w) cannot evolve further, under this control strategy, is when there is no remaining point on the possible set border with a higher realized fitness than the current point, with the current controls. This circumstance is termed an evolutionarily stable state and represents a selection equilibrium.

The policy of adjusting controls for exact sustainability ensures that as the population's phenotype moves along the border of the possible set, the $\lambda_1 = 1$ isocontour will follow it. The joint demographic and selection equilibrium occurs at the location where that isocontour has the same slope as the border of the possible set. At that location, if the border of the possible set is less concave than the hyperbolic $\lambda_1 = 1$ isocontour, all points in the (R_n, R_w) space with higher fitness, for this set of controls, are outside the possible set. Then, as long as the controls remain fixed, there is no available alternative phenotype that can invade and spread in the population, so the current (R_n, R_w) is an evolutionarily stable state.

Interestingly, for a defined harvest policy, the condition of no available phenotype capable of achieving a higher net growth rate, under these controls, corresponds to the condition of no available phenotype associated with a higher sustainable harvest under these controls. This perspective can be used to convert the search for the selection equilibrium to a relatively simple operations research problem of finding the location on the possible set border where, with harvest adjusted locally for exact replacement, the harvest is maximized.

General solution for the selection equilibrium

Imagine that the border of the possible set is described by some generalized, continuous, differentiable function:

$$R_w = \psi(R_n) \quad (20)$$

Imagine further that the harvest selectivity s and the broodstock withdrawal rates F_w and F_n are fixed by policy, so only the base harvest rate H_n need be considered directly for adjustment decisions as the system evolves: the broodstock withdrawal rates remain fixed, and the changes in H_w will follow from the adjustment in H_n. Then, the pre-

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ceding analysis provides us a direct mathematical strategy to solve for the selection equilibrium.

The selection equilibrium will occur for the value of R_a where the first derivative of the function for the possible set border, eq. 20, equals the first derivative of the $\lambda_1 = 1$ isocontour, eq. 14, where we substitute eq. 16 for H_w and eq. 17 for H_a, leaving an expression with only the one unknown, R_a, and the fixed control parameters s, F_w, and F_a. Alternatively, we may substitute eq. 20 for R_w in eq. 17, leaving an expression with only H_a and R_a as variables, and solve for the R_a that maximizes H_a. With either strategy, solution will depend on the specifics of the function ψ, namely the configuration of the possible set border. With a solution for the equilibrium R_a^e in hand, the solution for R_w^e is obtained with eq. 20.

Illustration for a linear possible set

If the possible set is more or less linear over the region of interest, it will be fully characterized by a slope α, and knowledge of the phenotype $R_{(a,0)}$, $R_{(w,0)}$ of the original population after the hatchery program was instituted but before the selection response. Then the intercept b of the possible set border can be solved with eq. 15. With a linear possible set, the derivative of eq. 20 is simply α, and the rest of the solution for the selection equilibrium follows.

To explore the response to the intensity of supplementation using hatchery progeny born of natural-origin broodstock, the surface of the equilibrium natural spawning fitness R_w^e may be plotted as a function of the possible set slope α and F_w, the rate of withdrawing broodstock from the naturally spawned adults, for a reference case where the natural spawning replacement rate of the preselection population was $R_{(w,0)} = 1$ and where the hatchery spawning replacement rate of the preselection population was $R_{(a,0)} = 5$, the rate of withdrawing broodstock from adults of hatchery spawning origin is fixed at $F_a = 0$, and the harvest is totally selective, $s = 1$, so only fish of hatchery spawning origin are harvested (Fig. 1, thick contour lines). We see that, depending on the value of the slope α and the intensity of the demographic contribution of the hatchery production (as measured by F_w), the resulting selection equilibrium can occur at a natural spawning fitness that is considerably depressed, going as low as about a 50% reduction in the upper right-hand corner of the plot. But there are also regions, below and to the left of the $R_w^e = 1$ contour, where there is no selection response to low levels of supplementation with hatchery progeny from exclusively natural-origin broodstock because of initial disequilibrium.

The choice of natural spawning replacement just at 1 for the preselection condition in the reference case reflects the motivation for supplementation as a means hopefully to ameliorate the effects of hatchery production influence on a weak stock that is of conservation concern. For high values of natural spawning replacement, there would be no sound reason for supplementation. We may explore a reasonable range of values for the preselection natural spawning replacement rate by plotting for each such value the contour where the first selection response occurs (the contour line where the selection equilibrium just equals the initial fitness). We see (from the thin contour lines in Fig. 1) that this family of first-response contours are gradually displaced from the first-response contour in the base case, and the general pattern persists. For all cases graphed, the depression of natural spawning fitness in the upper right-hand corner of the plane (intense supplementation, shallow trade-off between fitness in the two phases) corresponded to a loss of about 50% of the preselection natural spawning fitness.

To explore the response to use of hatchery spawned adults as broodstock, the surface of the equilibrium natural spawning fitness R_w^e may be plotted as a function of the possible set slope α, and F_a, the rate of withdrawing broodstock from the hatchery spawned adults, under conditions where the natural spawning replacement rate of the preselection population was $R_{(w,0)} = 1$, the hatchery spawning replacement rate of the preselection population was $R_{(a,0)} = 5$, the rate of withdrawing broodstock from adults of wild spawning origin is fixed at $F_w = 0.5$, and the harvest is totally selective, $s = 1$, so only fish of hatchery spawning origin are harvested (Fig. 2). This extends the previous reference scenario (Fig. 1) into the F_a dimension: with $F_a = 0$, the second scenario intersects the first scenario at $F_w = 0.5$. We note that as F_a, the broodstock withdrawal rate of hatchery-spawned fish, increases, the equilibrium natural spawning fitness decreases faster than linearly, going to zero if the broodstock withdrawal rate gets too high. Furthermore, for a steep enough possible set border, the response to increasing F_a is a knife-edge, an incipient fold catastrophe, going from no response to runaway selection when a critical broodstock withdrawal rate is exceeded.

In this particular scenario, defined by a broodstock withdrawal rate of naturally spawned fish fixed at $F_w = 0.5$, harvest selectivity fixed at $s = 1.0$, and a preselection phenotype consisting of a natural spawning replacement rate of $R_{(w,0)} = 1$, and a preselection hatchery spawning replacement rate of $R_{(a,0)} = 5$, the potential for abrupt transition to runaway selection response begins at possible set slopes steeper than $\alpha = -0.2$, at which slope the runaway selection response begins when the broodstock withdrawal rate of hatchery spawned fish reaches $F_a = 0.5$. We may therefore characterize the catastrophe potential for this scenario by the location in the (F_a, α) plane of the singular point, which is the shallowest slope α and the lowest value of hatchery fish broodstock withdrawal F_a where runaway selection first occurs. This is seen, in this scenario, at the point ($F_a = 0.5$, $\alpha = -0.2$) where the contours of equilibrium natural spawning fitness all converge. We may explore the range of parameter values for which this qualitative pattern of catastrophic response applies by asking how the location of the singular point moves in response to changes of the parameter values that define the scenario.

Generality of the pattern of catastrophic response

The results of the exploration of different scenarios may be displayed by graphing the location of the singular point in the (F_a, α) plane as a function of the parameter values defining the scenario (Fig. 3). The axes in this plot are F_a and α. The vertical contour lines are labelled according to the value of the broodstock withdrawal rate of naturally spawned fish F_w fixed by policy for that scenario. The oblique contour lines are labelled according to the value of

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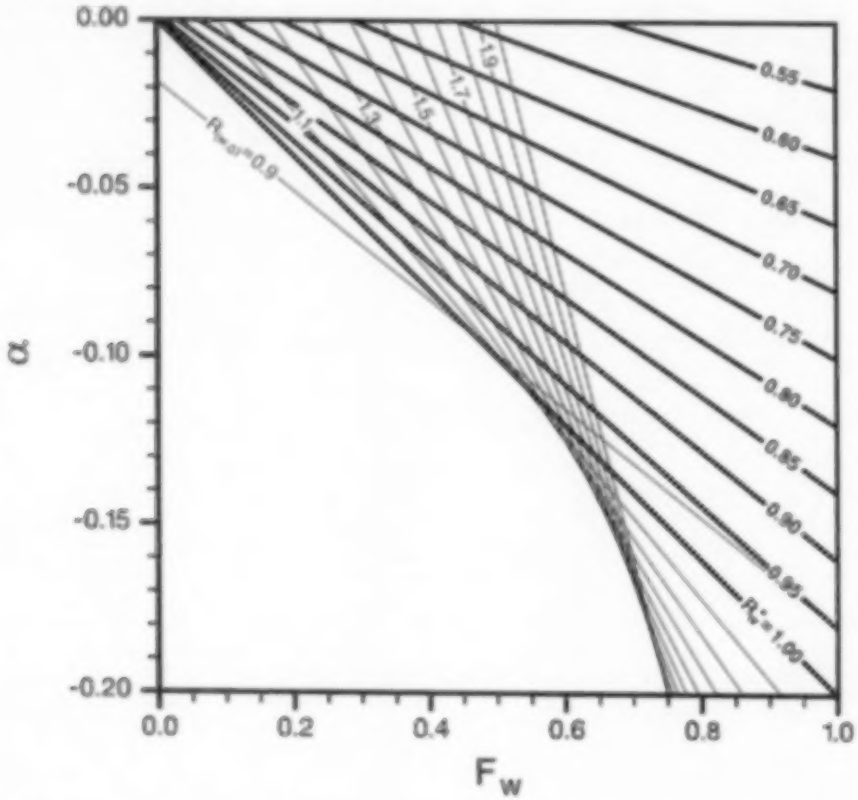
Fig. 1. Equilibrium natural spawning replacement rate R_n^e (thick contour lines) as a function of the slope of the possible set border α and the broodstock withdrawal rate of naturally spawned fish F_w for the reference case of the preselection phenotype having a natural spawning replacement rate R_n of 1, a hatchery spawning replacement rate R_h of 5, and 100% harvest selectivity ($H_w = 0$) and no taking of hatchery-spawned fish for broodstock and assuming a linear possible set border, truncated at the initial phenotype. In the portion of parameter space below and to the left of the R_n^e contour corresponding to the initial natural spawning replacement rate (in this case, $R_n^e = 1$), there is no selection response because of initial disequilibrium. The thin contour lines show the locations of first selection response for a family of scenarios that duplicate the reference case except for differing values of the preselection natural spawning replacement rate $R_{n,(0)}$ ranging from a low of 0.9 to a high of 2. The $R_{n,(0)} = 1$ contour is not visible because it is the same as the $R_n^e = 1$ contour for the case $R_{n,(0)} = 1$.



the preselection hatchery replacement rate R_h for that scenario. The intersections of F_w and R_h contours give the locations (in the (F_w, α) plane) of the singular point (where the catastrophic response of abrupt transition to runaway selection occurs) for that scenario, with the preselection natural spawning replacement rate still just at replacement, $R_{n,(0)} = 1$, and the harvest still completely selective, $s = 1$. We see that the pattern of catastrophic response persists over the portion of the parameter space shown, spanning the plausible range of values for preselection hatchery replacement rates and spanning the full range of possible policies for broodstock withdrawal rates of naturally spawned fish. The singularity appears where the broodstock removal rate of hatchery-spawned fish equals the broodstock removal rate of naturally spawned fish at a possible set slope value that becomes shallower for higher values of the preselection hatchery replacement rate.

The points in the plane that are labelled by letters inside a circle illustrate the response to changing the harvest selectivity or the preselection natural spawning replacement rate for the scenario. We see, then, that qualitatively, the pattern remains the same as all elements defining the scenario are perturbed.

Extension to a curved possible set border

Recall that the preceding illustrations presume a linear border for the possible set. This is certainly reasonable as a local approximation for portions of the possible set but is not likely to hold over the entire phenotype space.

If the possible set border is concave up in the vicinity of the initial equilibrium, this will lead to more strongly disjunctive selection responses as the system jumps between the respective adaptive peaks (Levins 1968). In our salmon example, such concavity of the possible set border could result from very distinct relatively successful life history alternatives whose intermediates are disadvantageous.

If the relative success of the alternative phenotypes varies more continuously, we would expect the possible set border, viewed at a larger than local scale, to be convex up. This

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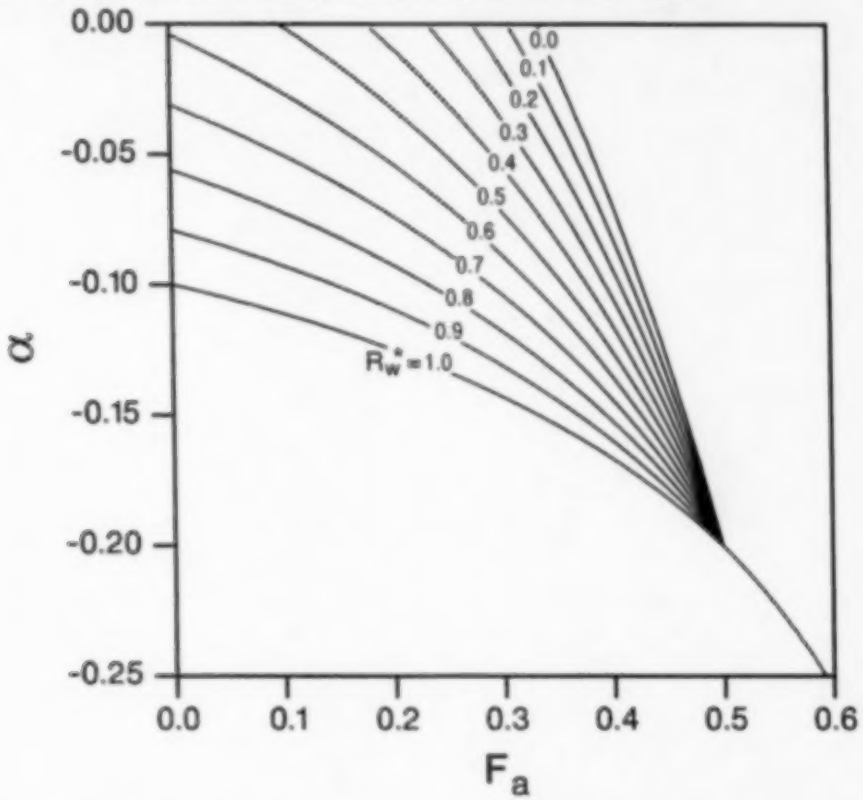
Fig. 2. Equilibrium natural spawning replacement rate R_w^e as a function of the slope of the possible set border α and the broodstock withdrawal rate of hatchery fish F_a for the reference case of the preselection phenotype having a natural spawning replacement rate R_w of exactly 1, a hatchery spawning replacement rate R_a of 5, a fixed rate of 0.5 for F_w, the broodstock withdrawal of naturally spawned fish, 100% harvest selectivity against taking wild fish, and assuming a linear possible set border truncated at the initial phenotype. In the portion of parameter space left of the $R_w^e = 1$ contour, there is no selection response because of initial disequilibrium. In the portion of parameter space to the right of the $R_w^e = 0$ contour, there is runaway selection to zero natural spawning fitness. At possible set slopes steeper than $\alpha = -0.2$, the transition from no response to runaway selection is knife-edged.



corresponds to the trade-off between fitness in the two environments varying according to the degree of displacement from the preselection state. In this situation, the first increments of new adaptation to the hatchery phase exact a small cost in natural phase fitness, but successive increments of hatchery adaptation exact increasingly higher costs to natural phase fitness. From the general experience with systematic animal breeding, a convex up possible set border would be typical for quantitative traits that both contribute positively to overall fitness.

The consequences of a convex up possible set border for the selection equilibrium that we have been investigating cut both ways. On the one hand, as supplementation proceeds in intensity, the moving selection equilibrium will encounter a progressively steeper slope on the possible set border as the natural spawning fitness declines, corresponding to a cost–benefit structure that becomes less conducive to further selection in that direction. As illustrated for the linear case (Figs. 1 and 2), a steeper slope α causes the selection equilibrium to occur at a smaller degree of depression of natural spawning fitness, all other things being equal. On the other hand, in the scenarios with hatchery-spawned fish included in the broodstock, as the slope α crosses some threshold, the selection dynamics encounter the knife-edge transition from no response to runaway selection as the fraction of hatchery-spawned fish taken for broodstock increases.

To see how these two forces play out, a numerical investigation was conducted for a family of possible sets where the curvature describes a segment of a circle and the amount of curvature is quantified in terms of the radius (in units of replacement rate). With the parameter α now characterizing the slope of the possible set border at the preselection state, the new equilibrium natural spawning fitness can again be shown as a function of that initial slope and the rate of withdrawal of hatchery-spawned fish for broodstock, with other aspects of the scenario (including the curvature) fixed (Fig. 4). One such scenario, corresponding in all other respects to a previous base case (initial $R_a = 5$, initial $R_w = 1$, $F_w = 0.5$, $s = 1$) but with the possible set border now having a radius of curvature of 40, shows, as expected, a reduction in the degree of depression of natural spawning fitness, compared with the linear case, over regions of the parameter space where the response to increasing supplementation is continuous, but a transition to runaway selection terminating

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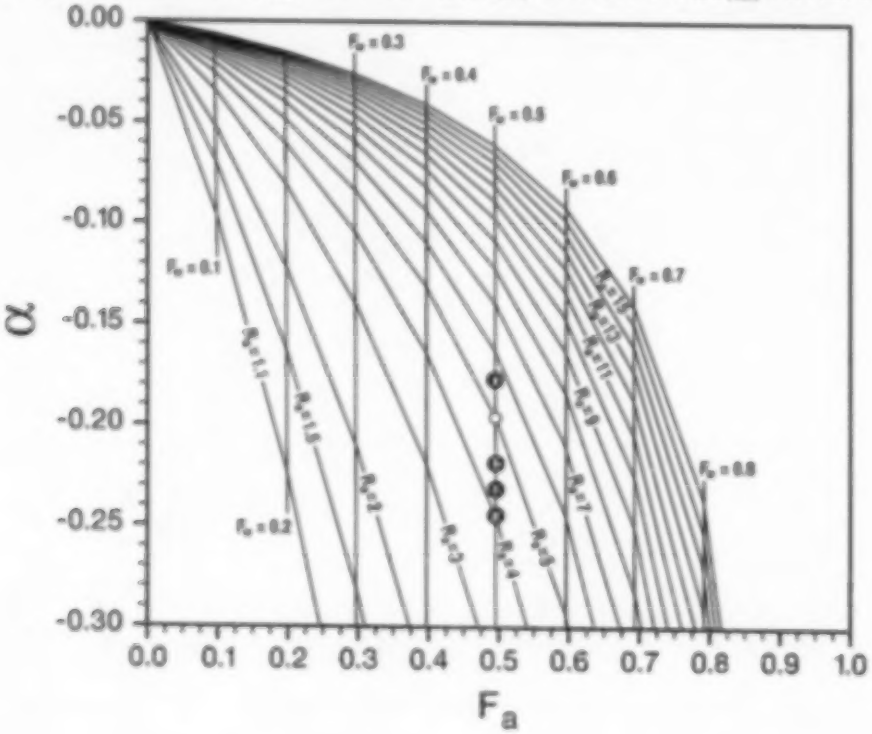
Fig. 3. Location in the (F_w, α) plane of the point where the knife-edged transition to runaway selection just begins as function of broodstock withdrawal rate of naturally spawned fish F_w (vertical contour lines) and preselection hatchery replacement rate R_a (oblique contour lines) for a family of scenarios where the preselection natural spawning replacement rate is exactly at replacement, $R_{(a,0)} = 1$ and harvest selectivity is absolute, $s = 1$. The points labelled with letters on the $F_w = 0.5$ contour line show the new location of the singular point as a result of perturbing the harvest selectivity and the preselection R_a while keeping $R_{(a,0)} = 5$ compared with the base case (open circle): a, $R_{(w,0)} = 1.1$ and $s = 1$; b, $R_{(w,0)} = 0.9$ and $s = 1$; c, $R_{(w,0)} = 1$ and $s = 0.9$; d, $R_{(w,0)} = 0.9$ and $s = 0.9$.



in zero natural spawning fitness still occurs for sufficiently high rates of supplementation, and a transition to a knife-edge response still occurs for a sufficiently steep trade-off slope. The locations of the singular point for a range of values of curvature of the possible set border are also shown in this graph, confirming that the general pattern continues to hold and that the effect of curvature is gradual.

Properties of a more restrictive supplementation protocol

The broodstock withdrawal rate of hatchery-spawned fish emerges as the critical control in the preceding analysis where exceedances of critical values in this parameter could lead to runaway selection and abrupt transitions to runaway selection. What if the supplementation protocol forbade any "recycling" of hatchery-spawned fish into the next generation's broodstock, as has in fact been recommended by some oversight panels (e.g., Regional Assessment of Supplementation Project 1992).

With the restrictive protocol $F_a = 0$ and $s = 1$, the equations simplify, since $N_{aa} = 0$ and $H_w = 0$. The condition for demographic equilibrium, eqs. 17–19, then reduces to

$$(21) \quad H_a = 1 + \frac{R_w(1 - F_w) - 1}{R_a R_w F_w}$$

and c, the fraction of naturally spawned fish on the natural spawning ground, is obtained from this and the simplification of eqs. 10–12 as

$$(22) \quad c = R_w(1 - F_w)$$

The equation for a linear segment of the possible set border is still given by eq. 15. Consider the segment of the possible set border that includes the preselection phenotype, so the intercept b of eq. 15 can be back-calculated from the preselection phenotype. In that neighborhood, exact replacement for any given value of the supplementation policy F_w is achieved by adjusting the harvest rate H_a to satisfy eq. 21.

To find the selection equilibrium as the phenotype (R_a, R_w) on the possible set border that maximizes the sustainable harvest with this level of supplementation, we first solve eq. 15 for R_a and substitute this for R_a in eq. 21, obtaining the function for sustainable harvests on the possible set border as

$$(23) \quad H_w = 1 + \frac{\alpha(R_w(1 - F_w) - 1)}{F_w R_w(R_w - b)}$$

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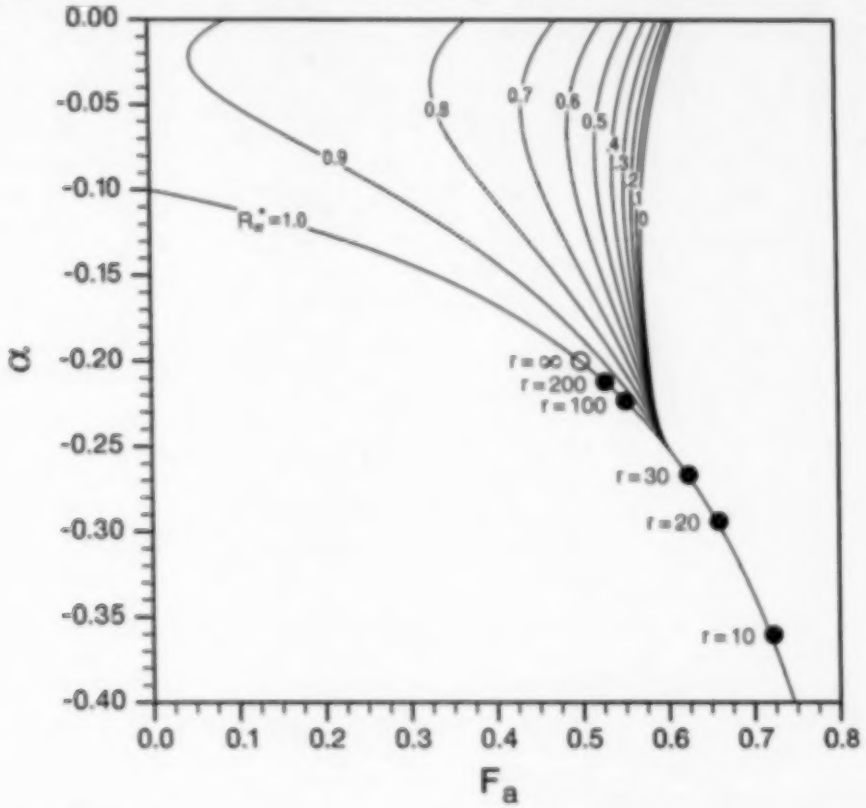
Fig. 4. Equilibrium natural spawning replacement rate R_w^e as a function of the initial slope of the possible set border α and the broodstock withdrawal rate of hatchery fish F_a for the reference case of the preselection phenotype having a natural spawning replacement rate R_w of exactly 1, a hatchery spawning replacement rate R_a of 5, a fixed rate of 0.5 for F_w, the broodstock withdrawal of naturally spawned fish, 100% harvest selectivity against taking wild fish, and assuming a possible set border that is a segment of a circle truncated at the initial phenotype with slope α at the initial phenotype and with radius 40. In the portion of parameter space left of the $R_w^e = 1$ contour, there is no selection response because of initial disequilibrium. In the portion of parameter space to the right of the $R_w^e = 0$ contour, there is runaway selection to zero natural spawning fitness. At initial possible set slopes steeper than $\alpha = -0.25$, the transition from no response to runaway selection is knife-edged. The labelled points show the location where the transition to knife-edged response just begins for other values of r, the radius of curvature of the possible set border.



Taking the first derivative with respect to R_w:

$$(24)\quad \frac{dH_a}{dR_w} = \frac{-\alpha}{F_w(R_w(R_w - b))^2}(R_w^2(1 - F_w) - 2R_w + b)$$

and solving for the value of R_w where the first derivative is zero to find the maximum, we obtain the quadratic

$$(25)\quad (R_w^e)^2(1 - F_w) - 2R_w^e + b = 0$$

the roots of which are

$$(26)\quad R_w^e = \frac{1 \pm \sqrt{1 - (1 - F_w)b}}{1 - F_w}$$

Substituting eq. 22 into eq. 26 and rearranging gives

$$(27)\quad c = 1 \pm \sqrt{1 - (1 - F_w)b}$$

so only the negative root has physical meaning, since c must lie between 0 and 1. Therefore, the solution for the naturally spawning intrinsic net replacement rate at any interior selection equilibrium is given by eq. 26 with the negative square root.

Taking the first derivative with respect to the broodstock withdrawal rate, we obtain

$$(28)\quad \frac{dR_w^e}{dF_w} = \frac{1}{1 - F_w}\left(R_w^e - \frac{b}{2\sqrt{b(1 - F_w)}}\right)$$

Substituting from eq. 26 for the square root term and then substituting from eqs. 22 and 15, eq. 28 becomes

$$(29)\quad \frac{dR_w^e}{dF_w} = \frac{(R_w^e)^3}{2\alpha R_a}$$

All of the terms in the above expression are positive, except for α, so we have that the equilibrium natural spawning fitness necessarily declines with increasing supplementation at selection equilibrium. Once a selection response to supple-

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mentation starts, increasing the level of supplementation will continue to depress the natural spawning fitness further.

The only possible singularities in eq. 29 would occur when α or R_n^e is zero. If the initial hatchery replacement rate was above 1, $R_n^e = 0$ cannot arise (and if the initial hatchery replacement rate is below 1, there is no point to using the stock for supplementation). A zero value for α implies that adaptation to the hatchery phase exacts no cost in natural spawning adaptation, which might be true for the first small increments of domestication selection but is implausible thereafter. Therefore, unlike the response to increases in use of hatchery-origin fish for broodstock, increases in the broodstock withdrawal rate of natural-origin fish cannot give rise to runaway selection in the strict supplementation protocol that we are considering here. This gives substance to recommendations that a strict supplementation protocol based on broodstock of naturally spawned origin is preferable to one that recycles hatchery-origin fish.

Further, we observe a central role for α, the slope of the border of the possible set, in determining the rate of decline of natural spawning fitness with increasing supplementation. The steeper the slope of the possible set border in the vicinity of the current selection equilibrium, the smaller will be the selection response to increased supplementation.

For the extreme case of $F_w = 1$, which will constitute a worst case from the standpoint of reduction of natural spawning fitness, under the strict supplementation protocol, the solution for sustainable selective harvest, eq. 21, reduces to

$$(30) \quad H_n = 1 - \frac{1}{R_n R_w}$$

Rearranging eq. 15 to substitute for R_n in terms of R_w on the possible set border gives

$$(31) \quad H_n = 1 - \frac{\alpha}{R_w^2 - bR_w}$$

which we solve for the R_w^e, the selection equilibrium, finding the value that maximizes the sustainable harvest by setting the first derivative to zero:

$$(32) \quad \frac{dH_n}{dR_w} = \frac{\alpha(2R_w - b)}{(R_w^2 - bR_w)^2}$$

so

$$(33) \quad R_w^e = \frac{b}{2}$$

Substituting for b, the value back-calculated from eq. 15 with the preselection phenotype ($R_{n,0}$, $R_{w,0}$) gives

$$(34) \quad R_w^e = \frac{R_{w,0} - \alpha R_{n,0}}{2}$$

Since the slope α must be nonpositive, the worst case obtains for zero slope, where

$$(35) \quad R_w^e = \frac{R_{w,0}}{2}$$

In the worst case, then, half of the preselection natural spawning fitness is sacrificed at the selection equilibrium (consistent with the appearance of the particular scenario of Fig. 1). This fitness loss is considerable but still better than the worst case when allowing hatchery-origin fish to be used for broodstock, for with the latter system, the natural spawning fitness can be depressed to zero.

The protection against runaway selection when no hatchery fish are used as broodstock is intuitive enough, and it is part of the theoretical attraction of the strict protocol as defined here. The requirement that all of the hatchery broodstock be withdrawn from the naturally spawned component of the adult run ensures that naturally spawned ancestry will make up at least 50% of the pedigree at replacement. This floor on the frequency of the natural spawning phase guarantees a minimum magnitude of selection pressure to maintain some degree of natural spawning fitness. This will "contain" the domestication selection to an intermediate level.

Discussion

Integrated hatchery breeding programs have been proposed as a possible solution to the problems posed by the negative effects of hatchery stocks on the wild stocks with which they interact. Once the integrated breeding program has been in place, the wild stock will no longer be distinct, so there is no point to asking whether the hatchery phase of the integrated population has a negative effect on the fitness of the natural spawning phase. This does not, however, preclude comparison of the characteristics of the original wild stock, before it came under hatchery influence, with the characteristics of the population that results from the integrated hatchery production. The present modelling analysis conducts such a comparison, where the characteristic of interest is the intrinsic replacement rate realized from natural spawning.

The adverse selection phenomena are the result of selection for traits that are adaptive in the hatchery-reared life cycle but are not optimal for a natural spawning life cycle. A significant component of the selection during the hatchery-reared life cycle may occur as life history selection after release, acting on life history consequences of the individual's state at release. Thus, strong selection, different from the selection on natural spawning fish, can occur during the course of a hatchery-reared generation, even if mortality within the hatchery is slight. Assuming that the wild population's original life history is at an adaptive peak, other selection on life history traits will move the phenotype off that peak, diminishing performance under conditions experienced by the wild population.

By representing the available phenotypes as a fitness set characterized by biologically possible combinations of hatchery replacement rate and natural spawning replacement rate, and combining this with the demographic model of the integrated production system, the analysis here has obtained a closed-form solution for the selection equilibrium that results. We find that integrated breeding has the potential for large depression of natural spawning fitness, potential for runaway selection driving the natural spawning fitness to zero, and potential for a discontinuous knife-edge response

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to gradual increases in the contribution of hatchery-spawned fish to the hatchery production of the integrated population.

The previous literature on evolutionary models of integrated production shows a potential for selection to reduce natural spawning fitness (Adkison 1995; Lynch and O'Hely 2001; Ford 2002). The present analysis generalizes and extends those results, showing a spectrum of selection responses, including runaway selection and a knife-edge response to increasing supplementation, and revealing the parameter ranges for which they occur. All of the models are in agreement that a reduction in natural spawning fitness is possible and that the reduction could be large, which lends credence to the concerns about large-scale implementation of integrated production programs and raises grave questions about the suitability of supplementation as a conservation measure for weak stocks that are still self-sustaining (Goodman 2004).

The capacity of the model developed here to solve explicitly for the selection equilibrium as a function of the management controls motivates us to inquire whether we can, with present knowledge, reasonably predict, quantitatively, the expected amount of depression of natural spawning fitness in a defined integrated breeding program.

The model of demographic equilibrium used here is sufficiently specified by six straightforward quantities: adult to adult replacement rates, harvest rates, and broodstock removal rates for both the hatchery spawned and naturally spawned fish. These are readily measurable, and while they have not all been measured systematically in well-controlled actual implementations of integrated production, we have enough experience with such measurements to know the range of values to expect for the initial condition (before selection has proceeded to a substantial extent).

The model for the selection equilibrium requires one more term, α, describing the negative correlation, at a selection limit, between natural spawning and hatchery spawning replacement rates. This term reflects an aspect of the set of biological possibilities for the stock. We have found that the results for any particular scenario examined with the model are strongly affected by this term. In contrast with the demographic quantities in the model, this trait correlation has never been measured for salmon in an integrated breeding program, and in fact, except for theoretical assurance of a negative correlation, we do not have a basis for expecting values in one range of magnitudes or another across the spectrum of values that give rise to quite different quantitative results for the selection equilibrium.

There are a modest number of stocks for which natural spawning and hatchery spawning replacement rates have both been measured. The collection of such empirical (R_n, R_w) pairs would indicate what the range of possibilities may be for a range of stocks over a range of environments, but the slope of the border of this collection of points is not the slope of the trade-off within a given stock for a given environment, and it is the latter that we need to characterize α quantitatively.

The local magnitude of the trait correlation term could in principle be back-calculated from measurements of the demographic quantities at the selection equilibrium, if we had them. But deliberate integrated production is a relatively new idea, and systematic empirical measurements have never been undertaken to monitor the evolution of natural spawning replacement rates, over many generations, in a program following a defined protocol. Practically, such measurements would also need to be made over enough brood years to average out the effects of environmental variation. Nevertheless, if supplementation is going to be deployed on an "experimental" basis, these are the quantities that need to be measured for the experiment to be informative.

The modelling results from this paper show that this experiment has the potential for a significant downside. It would be prudent to proceed cautiously in implementing integrated production with careful experimental design and systematic monitoring to determine how frequent and severe the depression of natural spawning fitness really is. In particular, since the modelling shows that the depression of natural spawning fitness will increase with the magnitude of the hatchery contribution to total production, it would be good to determine empirically whether specific policy caps on the amount of hatchery contribution can limit the fitness erosion to a tolerable level.

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Interaction of dietary sodium chloride and waterborne copper in rainbow trout (*Oncorhynchus mykiss*): copper toxicity and sodium and chloride homeostasis

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Abstract: Juvenile rainbow trout (*Oncorhynchus mykiss*) maintained on either low sodium chloride (LS (control), 1.4% NaCl) or high sodium chloride (HS, 11% NaCl) diet were exposed to 55 μg·L^{-1} waterborne copper (Cu) for 28 days. Cu-exposed fish maintained on the LS diet exhibited 26% mortality, more than double (11%) that in fish maintained on the HS diet. Waterborne Cu exposure inhibited growth by 56% in fish maintained on the LS diet and by 35% in those maintained on the HS diet. Whole-body and tissue Na^+ levels, measured 6 h after feeding, were increased by exposure to HS diet and reduced by waterborne Cu exposure. Exposure to elevated waterborne Cu increased whole-body and tissue Cu levels, whereas exposure to HS diet decreased these levels. Moreover, whole-body and tissue Cu concentrations were consistently lower in Cu-exposed fish maintained on HS diet relative to those maintained on LS diet. Plasma Na^+ and Cl^- levels were elevated by HS diet exposure and reduced by waterborne Cu exposure, whereas plasma Cu levels were decreased and increased by exposure to HS diet and waterborne Cu, respectively. These results demonstrate that elevated dietary NaCl modulates Na^+ and Cl^- homeostasis and reduces accumulation and toxicity of waterborne Cu.

Résumé : Des jeunes truites arc-en-ciel (*Oncorhynchus mykiss*), gardées à un régime faible (témoin) en chlorure de sodium (LS, 1,4 % NaCl) ou à un régime élevé (HS, 11 % NaCl), ont été exposés à 55 μg·L^{-1} de Cu en solution aqueuse pendant 28 jours. Les poissons gardés à un régime LS ont souffert une mortalité de 26 %, plus du double de celle (11 %) des poissons gardés au régime HS. L'exposition au Cu en solution aqueuse réduit la croissance des poissons gardés au régime LS de 56 % et celle des poissons gardés à HS de 35 %. Les concentrations de Na^+ dans le corps entier et les tissus, mesurées 6 h après l'alimentation, augmentent après un régime HS et diminuent après une exposition au Cu en solution aqueuse. Une exposition aux concentrations élevée de Cu en solution aqueuse augmente les concentrations de Cu dans le corps entier et les tissus, alors qu'un régime HS les diminue. De plus, les concentrations de Cu dans le corps entier et les tissus sont toujours plus faibles chez les poissons gardés à un régime HS et exposés au Cu que chez les poissons gardés au régime LS et exposés au cuivre. Les concentrations plasmatiques de Na^+ et de Cl^- augmentent avec un régime HS et diminuent après une exposition au Cu en solution aqueuse; en revanche, les concentrations plasmatiques de Cu diminuent à un régime HS et augmentent après une exposition au Cu en solution aqueuse. Ces résultats montrent qu'une augmentation de NaCl dans le régime module l'homéostasie de Na^+ et de Cl^- et réduit l'accumulation et la toxicité du Cu en solution aqueuse.

[Traduit par la Rédaction]

Introduction

The uptake of metal ions through biological membranes is strongly influenced by metal ion chemistry, but quite often ions that have very different chemical characteristics compete for uptake sites on biological surfaces. For example, despite a lower valence, a larger ionic radius, and a much higher concentration of Na^+ in natural waters compared with Cu^{2+}, these ions compete for uptake sites on the freshwater fish gill epithelium (Erickson et al. 1996; Santore et al. 2001; Grosell and Wood 2002). This is made possible in part by the involvement of three factors facilitating Cu^{2+} uptake. First, Cu^{2+} possesses several potential pathways of uptake and transport because it has the ability to form a wide range of coordination complexes with many cellular entities (Bury et al. 2003). Second, the hydrated (aquo) form of Cu^{2+}

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is large, such that in solution its mobility is similar to that of Na^+ (Handy et al. 2002) and it can therefore compete for diffusion sites on membranes. Third, the affinity of Cu^{2+} for binding (uptake) sites on the gill surface is more that four orders of magnitude higher than that of Na^+ (Santore et al. 2001), effectively compensating for the lower concentration of Cu^{2+} in natural waters.

Interactions between Na^+ and Cu in freshwater fish have therefore been the focus of several toxicological and physiological studies. Early studies showed that acute exposure of fish to waterborne Cu reduces plasma Na^+ and Cl^- concentrations (McKim et al. 1970; Schreck and Lortz 1978; Stagg and Shuttleworth 1982) as a consequence of inhibition of branchial Na^+ and Cl^- uptake processes (Laurén and McDonald 1985, 1986). Copper competitively inhibits (decreased affinity or increased K_m) and noncompetitively inhibits (reduced maximum uptake rate or J_{max}) the basolaterally located sodium pump, Na^+/K^+-adenosine triphosphatase (Na^+/K^+-ATPase; Laurén and McDonald 1987*b*; Sola et al. 1995; Li et al. 1998), leading to the impairment of branchial ionoregulatory mechanisms. However, very recent evidence now suggests that Na^+/K^+-ATPase impairment may not be the only potential mechanism for toxic action of Cu; other Na^+ (and Cl^-) uptake/transport processes are also impacted. There are now indications that Cu affects the epithelial Na^+ channel (Grosell and Wood 2002; Handy et al. 2002; Pyle et al. 2003), carbonic anhydrase and sodium-proton exchanger (Grosell et al. 2002), and ammonia excretion (Wilson and Taylor 1993; Taylor et al. 1996; Beaumont et al. 2003). All these mechanisms of toxicity are directly or indirectly associated with Na^+ metabolism and affirm that interference with Na^+ homeostasis is indeed the fundamental mechanism of acute waterborne Cu toxicity (Laurén and McDonald 1985, 1987*a*).

During prolonged sublethal exposure to waterborne Cu, partial or complete recovery of ionoregulatory function occurs, in part because of an increase in Na^+/K^+-ATPase activity (Stagg and Shuttleworth 1982; Laurén and McDonald 1987*a*; McGeer et al. 2000). Moreover, induction of metallothionein and increased sequestration of Cu in the gill (Dang et al. 1999, 2000) and liver (e.g., McCarter and Roch 1984) reduces the circulating levels of Cu. A possible additional strategy for fish to compensate for Cu-induced branchial Na^+ losses is to increase uptake and (or) utilization of dietary Na^+, because in addition to meeting their Na^+ requirement through branchial uptake (Lin and Randall 1995; Sullivan et al. 1995; Wood 2001), freshwater fish derive variable amounts of Na^+ from the diet depending on prevailing conditions. For example, in winter when foraging activity is low, trout principally meet their Na^+ requirement from water, whereas in summer, dietary and waterborne Na^+ uptakes are of the same magnitude (Smith et al. 1989). Several recent studies have advanced the concept that freshwater fish can take advantage of dietary ion surplus to mitigate the toxicity of waterborne toxicants that inhibit branchial ion uptake (Kamunde et al. 2003; Pyle et al. 2003; Baldisserotto et al. 2004). Specifically, Kamunde et al. (2003) and Pyle et al. (2003) demonstrated that elevated dietary Na^+ reduced gill binding and unidirectional uptake of waterborne Cu in rainbow trout while simultaneously decreasing branchial Na^+ uptake. Although the reduction in uptake and gill binding of Cu essentially implies reduction in Cu toxicity, protection against waterborne Cu toxicity by dietary NaCl is yet to be unambiguously demonstrated.

The purpose of the present study was therefore fourfold. First, because the concentrations of Cu used in our previous studies did not result in toxicity, we sought to expose juvenile rainbow trout to concentrations of waterborne Cu that would induce demonstrable toxicity, e.g., mortality/reduced survival and (or) growth inhibition, in order to characterize the potential protective effect of elevated dietary NaCl. Second, we evaluated the effects of both elevated waterborne Cu and dietary NaCl on Na^+ and Cl^- homeostasis by measuring tissue and plasma Na^+ and Cl^- concentrations soon after feeding Cu-exposed fish either a low or a high NaCl diet to capture the effect of Cu exposure when the fish are likely actively regulating an upsurge of plasma Na^+ from dietary uptake. Third, we examined changes in plasma Na:Cl ratios to assess the effect of waterborne Cu and dietary NaCl exposure on interdependency of Na^+ and Cl^- homeostatic mechanisms. Fourth, we assessed the potential protective effect of dietary NaCl on the bioaccumulation of Cu in whole-body and key target tissues. Overall, by evaluating growth, survival, and changes in tissue, plasma, and whole-body Na^+, Cl^-, and Cu during a long-term experiment, we sought to illuminate indicators of chronic Cu toxicity in freshwater fish.

Materials and methods

Experimental fish

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Humber Springs Trout Hatchery, Mono Mills, Ontario, and acclimated to laboratory conditions in a single 500-L plastic tank for 3 weeks. Laboratory conditions included flow-through dechlorinated Hamilton municipal tap water (Na^+ 0.6, Ca^{2+} 1.0, Cl^- 0.7, Mg^{2+} 0.2, HCO_3^- 1.9, all in mmol·L^{-1}; dissolved organic carbon 3.0 mg·L^{-1}; hardness 120 mg·L^{-1} as $CaCO_3$; pH 7.8–8.0; temperature 12 ± 1 °C). Fish were maintained on 1.5% wet body weight daily ration of commercial granulated 1.0 grade dry trout pellet (Corey Feed Mills, Fredericton, New Brunswick). The commercial trout diet contained crude protein 54% (minimum), crude fat 19% (minimum), crude fiber 2% (maximum), Ca^{2+} 1.5% (actual), phosphorus 1.1% (actual), Na^+ 0.6% (actual), vitamin A 10 000 IU (international units)·kg^{-1} (minimum), vitamin D3 6000 IU·kg^{-1} (minimum), and vitamin E 400 IU·kg^{-1} (minimum). Measured concentrations of Cu in water and food were 2.81 ± 0.43 µg·L^{-1} (n = 28) and 9.6 ± 0.76 µg·g^{-1} (n = 6), respectively, and the mean fish weight obtained by bulk weighing of all the fish at the beginning of the experiment was 19.9 g (wet weight).

Experimental set-up

The experiment described here was part of a larger study comprising a complete 2 × 3 complete factorial design (two waterborne Cu levels and three dietary NaCl treatments) that also involved assessment of food consumption and preference during waterborne Cu exposure. Here, we focus on two treatments tailored to investigate the effect of dietary NaCl and chronic waterborne Cu exposure on sublethal toxicity, Cu accumulation, and Na^+ and Cl^- homeostasis. The results of the third treatment are the subject of a separate publication (S. Niyogi, C.N. Kamunde, and C.M. Wood, unpublished).

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Following laboratory acclimatization, fish were equally (n = 33–35) and randomly distributed into six 150-L experimental tanks. Exposure to 55 $\mu g \cdot L^{-1}$ waterborne Cu was achieved via a constant drip of 2.5 $mL \cdot min^{-1}$ of a stock solution containing 68.75 $mg \cdot L^{-1}$ Cu (as $CuSO_4 \cdot 5H_2O$; Fisher Scientific, Toronto, Ontario) from a Marriott bottle into a head tank receiving 2.5 $L \cdot min^{-1}$ of dechlorinated Hamilton tap water. Uniform distribution of Cu in the head tank was achieved by constant vigorous aeration. The head tank supplied two experimental tanks (plus one for the diet selection study) at a flow rate of 800 $mL \cdot min^{-1}$. Actual Cu concentration in the experimental tanks was 52.34 ± 4.71 $\mu g \cdot L^{-1}$ (n = 28), and measured Cu concentration in the control tanks was 2.81 ± 0.76 $\mu g \cdot L^{-1}$ (n = 28). Fish in one of the tanks receiving Cu-contaminated water were maintained on a HS diet, whereas those in the other were maintained on a LS diet. The third Cu-treatment tank was used for a diet selection study. Two control tanks (plus one for a diet selection study) were maintained on regular dechlorinated tap water, with fish in one of the tanks receiving HS diet and the other receiving LS diet.

Diets, fish maintenance, and sampling

The HS diet was made in-house by supplementing commercial trout chow to a nominal level of 10% NaCl (by weight) as previously reported (Kamunde et al. 2003; Pyle et al. 2003). Control (i.e., LS) diet was taken through the same processing procedure except that no NaCl was added. Actual Na^+ concentrations were determined from samples (n = 6) of each diet: LS diet, 0.24 ± 0.02 $mmol \cdot g^{-1}$ (1.4% NaCl), HS diet, 1.89 ± 0.17 $mmol \cdot g^{-1}$ (11% NaCl). The diets were kept at –20 °C until offered to the experimental fish at 1.5% wet body weight ration twice per day (in the morning and evening for a total daily ration of 3.0%). One hour after each feeding, undigested food was siphoned off to minimize leaching of Na^+ into the water. All the fish were bulk-weighed weekly to determine growth and the ration for the ensuing week. Fish (n = 6–10) were sampled from each of the four treatments on days 4, 7, 14, and 28. An initial sample of 10 fish was obtained from the holding tank immediately before the random separation of the fish among the tanks, thereby providing the day 0 (baseline) data.

At each sampling time, fish were killed with an overdose of neutralized tricaine methanesulfonate; blood samples were taken immediately by caudal puncture and centrifuged for 4 min at 10 000*g* to separate plasma, which was decanted into separate plastic centrifuge tubes. The fish were then rinsed in running double-distilled water, blotted dry, and weighed. Gills, liver, gut, kidney, and the rest of the carcass were subsequently dissected into preweighed scintillation vials. Before placing them into scintillation vials, gills were rinsed in running double-distilled water for 30 s while the gut tissues were emptied of their contents, thoroughly washed in running double-distilled water, and blotted dry. All tissues, including plasma, were stored at –20 °C until analyzed for total Na^+, Cl^-, and Cu as described below.

Analysis

All tissues were digested overnight with six times their volume of 1 $mol \cdot L^{-1}$ HNO_3 (trace metal grade; Fisher Scientific, Nepean, Ontario), and subsamples were pipetted into 2-mL centrifuge tubes and centrifuged for 4 min at 10 000*g*. The supernatants were then diluted appropriately with 1% HNO_3 and analyzed for total Na^+ and Cu concentrations. Total Cu concentrations were measured by graphite furnace atomic absorption spectrophotometry (GFAAS; Varian SpectrAA-220, Mulgrave, Australia) with a graphite tube atomizer (GTA-110) while total Na^+ concentrations were measured by flame atomic absorption spectrophotometry (FAAS; Varian SpectrAA-220FS, Mulgrave, Australia). Water and plasma samples were similarly analyzed for total Na^+ and Cu concentrations after appropriate dilution. In addition, plasma was analyzed for Cl^- by the protocol of Zall et al. (1956) adapted for a microplate reader. Analytical quality assurance and control were maintained using method blanks and sample duplicates and were validated with certified reference materials (National Research Council of Canada, Ottawa, Ontario).

Calculations

Fish condition factor (k) was calculated using the following formula:

$$k = 100(\text{weight} \times \text{length}^{-3})$$

Whole-body total Na^+ and Cu concentrations were calculated by dividing the sum of Na^+ or Cu contents (concentrations multiplied by weight) of all the tissues sampled plus the carcass by the sum of weights of all the tissues plus the carcass.

Statistical analysis

All Na^+, Cl^-, and Cu accumulation data were statistically analyzed using three-way analysis of variance (ANOVA, Statistica version 6.0) with time, dietary NaCl, and level of waterborne Cu exposure as independent variables. A two-way ANOVA with dietary NaCl and waterborne Cu exposure levels as independent variables was used to analyze the condition factor and proportional distribution data (day 28 only). The proportional distribution data were arcsine transformed before statistical analysis. Tukey's honestly significant difference (HSD) test was used to delineate differences in mean values and to test for interactions among the main factors. Mean values were considered different at $P < 0.05$. The assumptions of ANOVA, i.e., homogeneity of variances and normality of distribution, were tested using Bartlett and χ^2 tests. All data met these assumptions. Differences in mortalities between control and Cu-exposed fish were delineated using Pearson's χ^2 test.

Results

Mortality and growth

Exposure of juvenile rainbow trout to 55 $\mu g \cdot L^{-1}$ waterborne Cu for 28 days caused 11% mortality in the fish maintained on HS diet, significantly lower ($P < 0.05$, Pearson's χ^2) than the 26% mortality in fish maintained on LS diets. This mortality occurred mainly during the first week of Cu exposure. Cu-unexposed fish on either diet showed no mortality. Growth was severely impacted by Cu exposure ($F_{[1,76]} = 81.03$, $P < 0.0001$), with the fish maintained on the LS diet showing net weight loss relative to the starting weight (Fig. 1*a*). At day 28, relative to control group, growth was not significantly altered (–9%, $P > 0.05$, not significant) in

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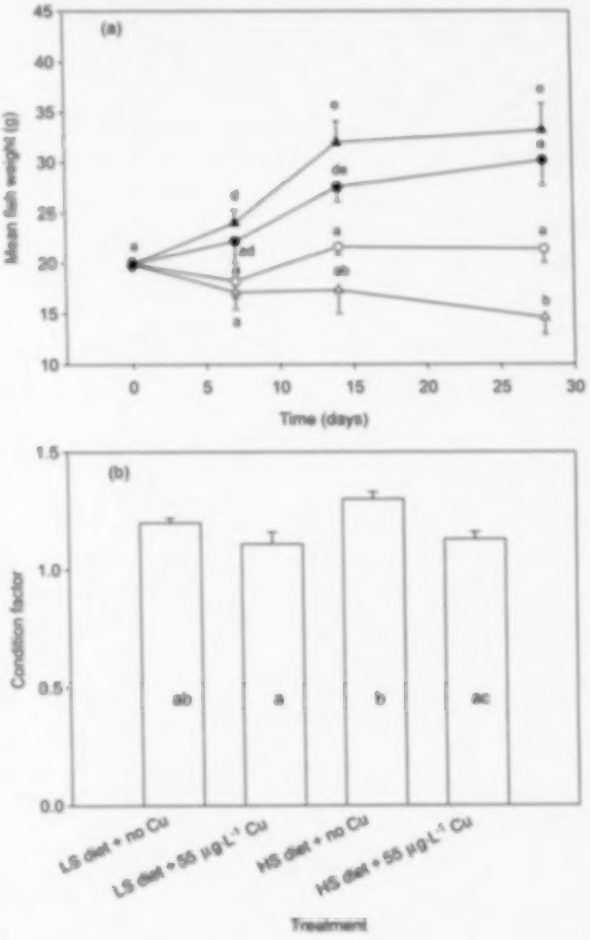
Cu-unexposed fish maintained on HS diet but was inhibited by 35% ($P < 0.05$) in Cu-exposed fish maintained on HS diet and by 56% ($P < 0.05$) in Cu-exposed fish maintained on LS diet. Time had a significant effect on growth ($F_{[2,76]} = 7.01$, $P < 0.005$), and there was a strong interaction between dietary NaCl and waterborne Cu ($F_{[1,76]} = 10.73$, $P < 0.005$), indicating that elevated dietary NaCl partly protected against the Cu-induced growth inhibition. There was also a significant interaction between time and waterborne Cu ($F_{[2,76]} = 3.98$, $P < 0.05$). Fish condition factor ranged from 1.10 to 1.30 on day 28 (Fig. 1*b*) and was adversely impacted by exposure to elevated waterborne Cu ($F_{[1,32]} = 12.37$, $P < 0.005$) but not by exposure to elevated dietary NaCl.

Fig. 1. Effect of exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to elevated waterborne Cu and dietary NaCl on (*a*) mean body weight and (*b*) condition factor. Weight gain was significantly impaired by elevated waterborne Cu exposure ($F_{[1,76]} = 81.03$, $P < 0.0001$), whereas condition factor was reduced by waterborne Cu ($F_{[1,32]} = 12.37$, $P < 0.001$) and increased by dietary NaCl ($F_{[1,32]} = 3.13$, $P < 0.05$). Points and bars with different letters are significantly different ($P < 0.05$). ▲, LS diet + no Cu; ●, HS diet + no Cu; △, LS diet + 55 μg·L^{-1} waterborne Cu; ○, HS diet + 55 μg·L^{-1} waterborne Cu.



Whole-body and tissue Cu accumulation

Exposure to elevated waterborne Cu increased whole-body Cu concentration ($F_{[3,101]} = 62.95$, $P < 0.0001$) by up to 80% (Fig. 2*a*) in the fish maintained on the LS diet relative to their controls, whereas elevated dietary NaCl significantly impeded whole-body Cu accumulation ($F_{[1,101]} = 18.01$, $P < 0.0001$). Whole-body Cu concentration generally increased with time ($F_{[3,101]} = 5.51$, $P < 0.05$), and there was significant interaction between dietary NaCl and waterborne Cu ($F_{[1,101]} = 5.92$, $P < 0.05$).

Organ/tissue Cu concentrations generally followed the same trend as for whole-body (Figs. 2*b*–2*f*). In the gill (Fig. 2*b*), elevated waterborne Cu exposure greatly increased the total Cu concentration ($F_{[1,101]} = 145.18$, $P < 0.0001$). Gill Cu concentration increased 2.5-fold in the Cu-exposed fish maintained on the LS diet relative to controls and had achieved a steady state by day 28. In contrast, dietary NaCl substantially impeded Cu accumulation in the gills ($F_{[1,101]} = 19.66$, $P < 0.0001$); thus Cu-exposed fish maintained on the HS diet showed only a 1.8-fold increase in Cu concentration on day 7, declining to more or less the control value by day 28. Overall, gill Cu concentration increased significantly with time ($F_{[3,101]} = 12.54$, $P < 0.0001$). Three-way ANOVA revealed significant interactions between time and dietary NaCl ($F_{[3,101]} = 4.77$, $P < 0.01$), time and waterborne Cu ($F_{[3,101]} = 5.46$, $P < 0.01$), and dietary NaCl and waterborne Cu ($F_{[1,101]} = 16.69$, $P < 0.0001$), as well as a three-way interaction between time, dietary NaCl, and waterborne Cu ($F_{[3,101]} = 3.21$, $P < 0.05$).

Of all tissues examined, the liver accumulated by far the greatest concentrations of Cu (Fig. 2*c*). Elevated waterborne Cu exposure significantly elevated the Cu concentrations ($F_{[1,101]} = 50.42$, $P < 0.0001$), whereas the HS diet reduced the accumulation of Cu ($F_{[1,101]} = 6.73$, $P < 0.05$) in the liver. A 40% reduction in Cu accumulation was observed in Cu-exposed fish maintained on HS diet relative to Cu-exposed fish maintained on LS diet (Fig. 2*c*). Overall liver Cu concentration had leveled off by day 14 of exposure in all Cu-exposed fish.

Kidney Cu accumulation (Fig. 2*d*) was only about 5% of that in the liver, and Cu concentrations were significantly increased by elevated waterborne Cu exposure ($F_{[1,101]} = 13.38$, $P < 0.005$). In addition, kidney Cu concentrations increased with time ($F_{[3,101]} = 4.93$, $P < 0.005$) but were significantly decreased by exposure to elevated dietary NaCl ($F_{[1,101]} = 10.49$, $P < 0.005$). There was significant interaction between dietary NaCl and waterborne Cu exposure ($F_{[1,101]} = 4.23$, $P < 0.05$) in determining Cu accumulation in the kidney.

The gut tissue (Fig. 2*e*) accumulated the next highest Cu concentrations relative to the liver, but overall these amounted to less than 15% of liver values. Elevated waterborne Cu exposure significantly elevated ($F_{[1,101]} = 29.23$, $P < 0.0001$), while dietary NaCl greatly impeded ($F_{[1,101]} = 11.87$, $P < 0.001$) the accumulation of Cu in gut tissues. There was significant interaction between dietary NaCl and waterborne Cu ($F_{[1,101]} = 10.31$, $P < 0.005$).

Overall, the carcass (Fig. 2*f*) contained the lowest concentrations of Cu of all of the tissues examined (0.35–

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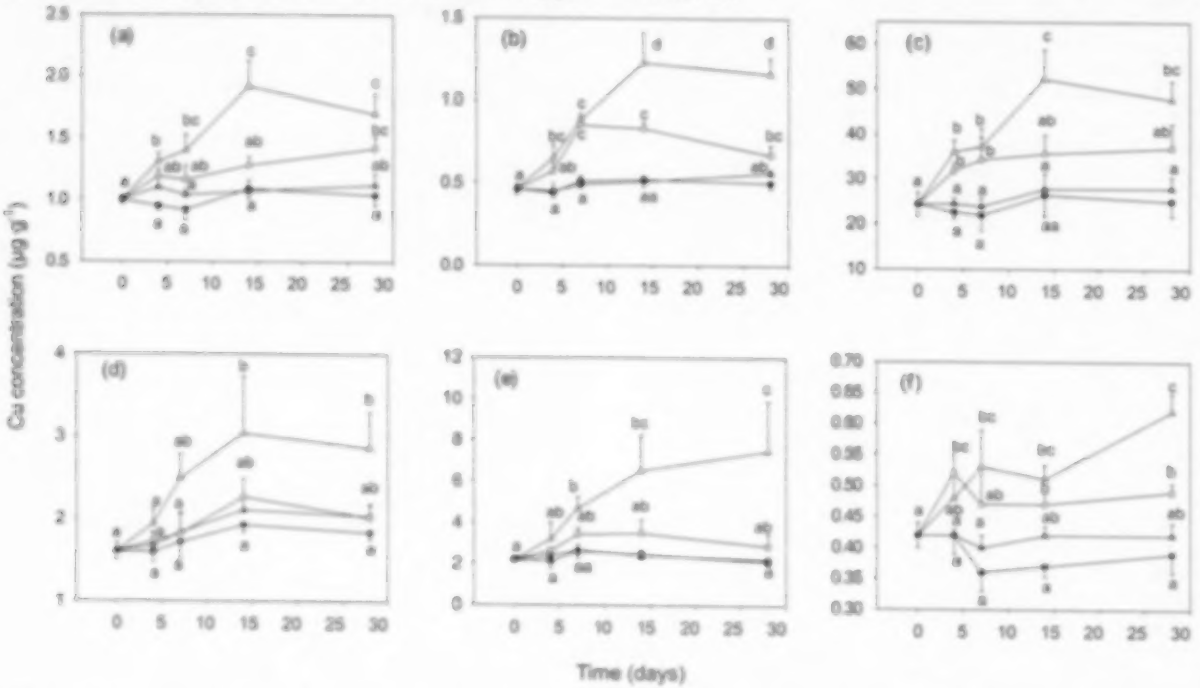
Fig. 2. Effect of elevated dietary NaCl and waterborne Cu exposure on accumulation of Cu in (*a*) whole body, (*b*) gill, (*c*) liver, (*d*) kidney, (*e*) gut, and (*f*) carcass. Elevated dietary NaCl impeded Cu accumulation, whereas elevated waterborne Cu increased Cu accumulation. Points on the same panel with different letters are significantly different ($P < 0.05$). ▲, LS diet + no Cu; ●, HS diet + no Cu; △, LS diet + 55 μg·L^{-1} waterborne Cu; ○, HS diet + 55 μg·L^{-1} waterborne Cu.



0.62 μg·g^{-1}). However, carcass Cu concentration was greatly increased by elevated waterborne Cu exposure ($F_{[1,101]}$ = 49.79, $P < 0.0001$), whereas elevated dietary NaCl significantly impeded accumulation of Cu ($F_{[1,101]} = 6.44$, $P < 0.05$).

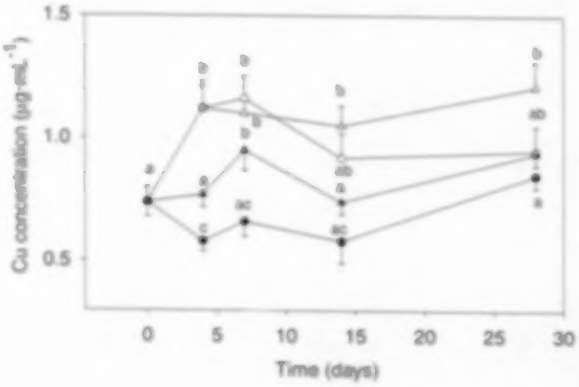
Plasma Cu concentrations

Plasma Cu concentrations (Fig. 3) were more tightly regulated than tissue Cu concentrations, varying only between 0.6 and 1.2 μg·mL^{-1}, but did respond to the experimental treatments. Plasma Cu concentrations were significantly elevated ($F_{[1,99]}$ = 23.42, $P < 0.0001$) and depressed ($F_{[1,99]}$ = 19.18, $P < 0.0001$) following exposure to elevated waterborne Cu and dietary NaCl, respectively. There were interactions between time and dietary NaCl ($F_{[3,99]}$ = 4.73, $P < 0.005$) and time and waterborne Cu ($F_{[3,99]}$ = 4.71, $P < 0.005$), as well as between dietary NaCl and waterborne Cu ($F_{[1,99]}$ = 5.83, $P < 0.05$).

Proportional distribution of Cu

Mass-weighted Cu distributions in gill, liver, kidney, gut tissue, and carcass on day 28 are shown for the various treatment groups (Fig. 4). For all groups, the liver contained most of the Cu (49%–58%), followed in decreasing order by the carcass (32%–37%), gut tissues (7%–12%), gill (1%–2%), and kidney (1%–2%). There was a significant effect of exposure to both elevated waterborne Cu ($F_{[1,32]}$ = 7.30, $P < 0.05$) and dietary NaCl ($F_{[1,32]}$ = 8.49, $P < 0.01$) on the contribution of the gill to whole-body Cu burden, with a significant interaction ($F_{[1,32]}$ = 11.32, $P < 0.01$) between the two factors. In addition, distribution of Cu in the gut tissues was

Fig. 3. Effect of elevated dietary NaCl and waterborne Cu exposure on plasma Cu concentrations. Elevated dietary NaCl decreased plasma Cu levels, whereas elevated waterborne Cu increased plasma Cu levels. Points with different letters are significantly different ($P < 0.05$). ▲, LS diet + no Cu; ●, HS diet + no Cu; △, LS diet + 55 μg·L^{-1} waterborne Cu; ○, HS diet + 55 μg·L^{-1} waterborne Cu.



significantly reduced by exposure to elevated dietary NaCl ($F_{[1,32]}$ = 4.33, $P < 0.05$) but was unaffected by exposure to elevated waterborne Cu. There was a significant interaction ($F_{[1,32]}$ = 5.06, $P < 0.05$) between waterborne Cu and dietary NaCl.

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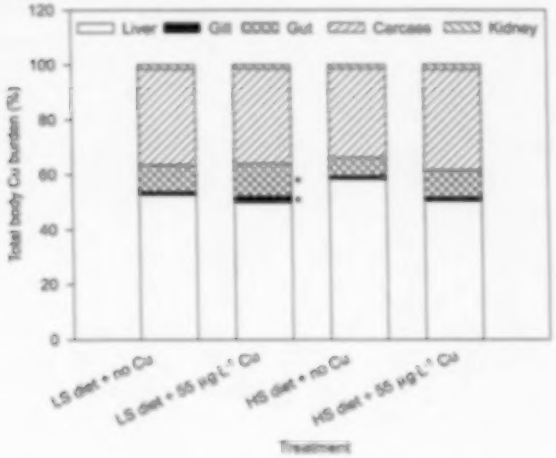
Fig. 4. Effect of elevated dietary NaCl and waterborne Cu exposure on proportional distribution of Cu in the main body compartments at day 28. Asterisk (*) indicates significantly different from controls ($P < 0.05$).



Whole-body and tissue Na^+ concentrations

Sodium concentrations were fairly uniform in all tissues examined, ranging from 35 to 90 μmol·g^{-1}. Exposure to elevated dietary NaCl significantly increased whole-body Na^+ concentrations ($F_{[1,101]} = 100.43$, $P < 0.0001$) from 50–53 μmol·g^{-1} in control fish maintained on the LS diet to 63–72 μmol·g^{-1} in Cu-unexposed fish and 57–64 μmol·g^{-1} in Cu-exposed fish (Fig. 5*a*). In contrast, elevated waterborne Cu exposure caused significant reductions in whole-body Na^+ concentrations ($F_{[1,101]} = 10.83$, $P < 0.005$). These changes became attenuated with time such that by day 28, differences occurred based on dietary NaCl treatment but not waterborne Cu exposure level.

Gill Na^+ concentration (Fig. 5*b*) increased significantly ($F_{[1,101]} = 56.35$, $P < 0.0001$) in response to the HS diet from 40–50 to 64–75 μmol·g^{-1} in Cu-unexposed fish and 52–72 μmol·g^{-1} in Cu-exposed fish. In contrast, elevated waterborne Cu exposure caused a significant decline in the gill Na^+ concentrations ($F_{[1,101]} = 4.62$, $P < 0.05$). Generally, the greatest effect of waterborne Cu exposure on Na^+ levels occurred in the fish maintained on the HS diet.

Liver Na^+ concentrations increased dramatically in response to the HS diet exposure ($F_{[1,101]} = 153.63$, $P < 0.0001$), with values ranging from 42–50 μmol·g^{-1} in the controls to 70–88 μmol·g^{-1} in the group maintained on HS diet (Fig. 5*c*). In contrast, elevated waterborne Cu exposure significantly decreased liver Na^+ concentrations ($F_{[1,101]} = 4.60$, $P < 0.05$).

In the kidney (Fig. 5*d*), dietary NaCl increased ($F_{[1,101]} = 25.63$, $P < 0.0001$) and waterborne Cu exposure reduced ($F_{[1,101]} = 5.91$, $P < 0.05$) Na^+ concentrations. There was a significant interaction between time, dietary Na^+, and waterborne Cu ($F_{[3,101]} = 6.51$, $P < 0.001$). Kidney Na^+ concentrations showed a "cross-over" effect in the two groups maintained on HS diet whereby the Na^+ concentration decreased dramatically to 39 μmol·g^{-1} early in the exposure in Cu-exposed fish maintained on the HS diet but subsequently increased to a level of 61 μmol·g^{-1} on day 28, the highest kidney Na^+ concentration recorded in the experiment.

For the gut (Fig. 5*e*), exposure to the HS diet elevated ($F_{[1,101]} = 47.65$, $P < 0.0001$) while exposure to elevated waterborne Cu decreased ($F_{[1,101]} = 7.08$, $P < 0.01$) Na^+ concentrations. As with the kidney, there was a cross-over effect resulting in the gut tissues of Cu-exposed fish maintained on the HS diet having the highest Na^+ concentration at day 28.

Carcass Na^+ concentrations (Fig. 5*f*) were also significantly elevated by exposure to elevated dietary Na^+ ($F_{[1,101]} = 83.64$, $P < 0.0001$) and decreased by elevated waterborne Cu exposure ($F_{[1,101]} = 9.50$, $P < 0.005$). These effects were greatest early in the exposure and became attenuated with time.

Plasma Na^+ and Cl^- concentrations

Plasma Na^+ concentrations (Fig. 6*a*) were significantly reduced by exposure to elevated waterborne Cu ($F_{[1,99]} = 20.54$, $P < 0.0001$) but significantly increased by elevated dietary NaCl ($F_{[1,99]} = 38.13$, $P < 0.0001$) and by time ($F_{[3,99]} = 25.09$, $P < 0.0001$). There were interactions between dietary NaCl and waterborne Cu ($F_{[1,99]} = 3.94$, $P < 0.05$) and among the three factors ($F_{[3,99]} = 14.14$, $P < 0.0001$). Plasma Cl^- concentrations (Fig. 6*b*) were significantly increased ($F_{[1,99]} = 52.01$, $P < 0.0001$) and decreased ($F_{[1,99]} = 11.42$, $P < 0.005$) by elevated dietary NaCl and waterborne Cu exposure, respectively. Na:Cl ratios (Table 1) ranged from 0.90 to 1.30 and were significantly lower ($F_{[1,99]} = 15.97$, $P < 0.0005$) in fish maintained on HS diet. These ratios, however, increased with time ($F_{[1,99]} = 11.80$, $P < 0.0001$).

Discussion

Mortality and growth

We achieved our objective of establishing quantifiable differences in toxicity whereby trout exposed to elevated waterborne Cu and maintained on the LS diet exhibited significantly higher mortality and greater growth inhibition relative to those maintained on the HS diet. Clearly, dietary NaCl protected fish against waterborne Cu toxicity. Although growth inhibition has been previously reported during waterborne Cu exposure in fish (e.g., Lett et al. 1976; Collvin 1985; Marr et al. 1996), ours is the first report of protection against the growth inhibitory effects of waterborne Cu exposure by elevated dietary NaCl in fish. This supports the proposition in our recent studies (Kamunde et al. 2003; Pyle et al. 2003) that reduction in the uptake rate and gill binding of Cu following elevated dietary NaCl exposure has implications for the toxicity of waterborne Cu in fish. The sustained growth retardation during chronic waterborne Cu exposure observed in the present study is in agreement with some studies (McKim and Benoit 1971; Buckley et al. 1982; Marr et al. 1996) but contrasts with others that reported recovery of weight gain to control levels with continued exposure (Drummond et al. 1973; Lett et al. 1976; Collvin 1985) or no effect of elevated waterborne Cu on growth (e.g., Taylor et al. 2000; McGeer et al. 2000, 2002).

Whole-body and tissue Cu and Na^+ concentrations

Cu-exposed fish maintained on HS diet had lower whole-body and tissue Cu concentrations relative to the Cu-exposed fish maintained on LS diet, consistent with the view that

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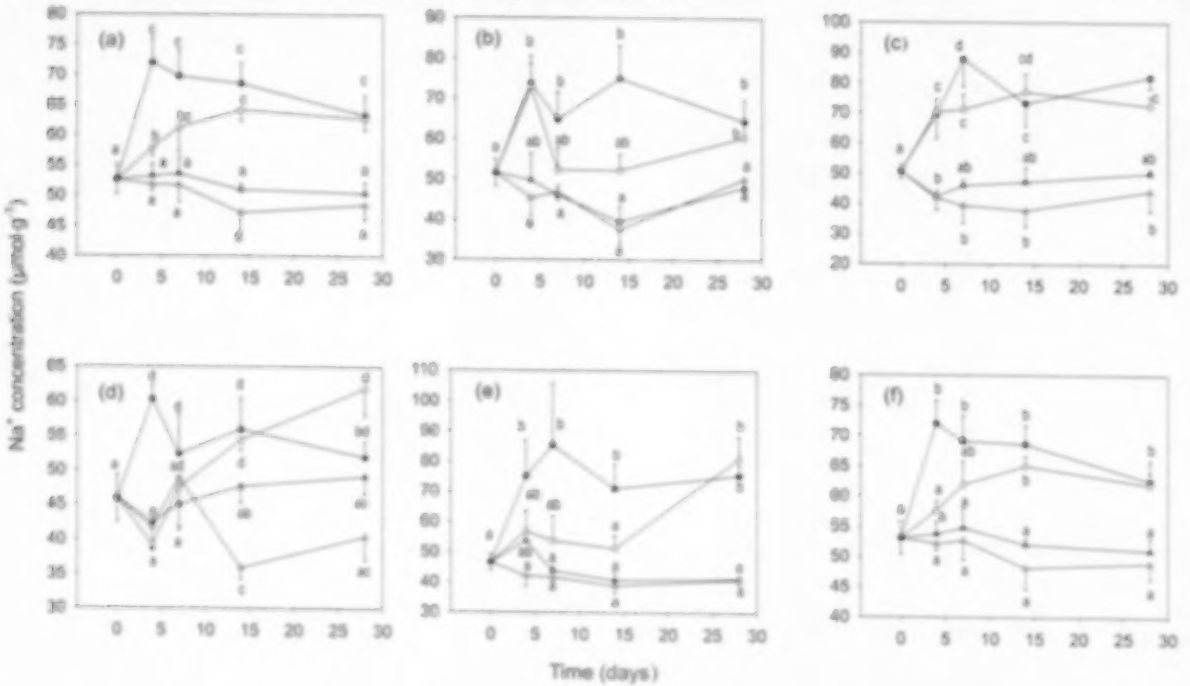
Fig. 5. Effect of elevated dietary NaCl and waterborne Cu exposure on total Na^+ concentrations in (*a*) whole body, (*b*) gill, (*c*) liver, (*d*) kidney, (*e*) gut, and (*f*) carcass. Elevated dietary NaCl increased the Na^+ levels, whereas elevated waterborne Cu reduced the Na^+ levels. Points in the same panel with different letters are significantly different ($P < 0.05$). ▲, LS diet + no Cu; ●, HS diet + no Cu; △, LS diet + 55 $\mu g \cdot L^{-1}$ waterborne Cu; ○, HS diet + 55 $\mu g \cdot L^{-1}$ waterborne Cu.



dietary NaCl impedes uptake and accumulation of Cu (Kamunde et al. 2003; Pyle et al. 2003). The efficacy of dietary NaCl in reducing Cu accumulation was extremely high in tissues such as the gill and gut in which Cu accumulation was reduced to concentrations equal to those of Cu-unexposed controls. The gill and gut tissues also showed interesting changes in proportional Cu distribution, which suggested that maintenance of fish on diets elevated in NaCl prevented imbalances in tissue Cu disposition. Whereas the contributions of the gill and gut tissues to whole-body Cu burden remained comparable to controls in Cu-exposed fish maintained on HS diet, both gill and gut tissues contributed significantly more to whole-body Cu burden in Cu-exposed fish maintained on LS diet. The significant accumulation of Cu in gut tissues of Cu-exposed fish maintained on LS diet was surprising because waterborne Cu rarely accumulates in gut tissues and gut Cu accumulation has been interpreted as diagnostic of dietary exposure (Handy 1992; Kamunde et al. 2001; Clearwater et al. 2002). It is possible that Cu accumulation in the gut was from stress-induced drinking, water ingested along with the food, and (or) from Cu-enriched bile via the hepatobiliary axis rather than directly from branchial uptake.

Unlike our recent study (Kamunde et al. 2003) in which minimal changes in tissue Na^+ concentrations occurred, the present study revealed highly significant elevations in whole-body and tissue Na^+ levels in response to elevated dietary NaCl exposure. This is probably because Na^+ analyses were performed on fish sacrificed 6 h after feeding, a period when fish tissues likely experienced an upsurge of tissue Na^+ concentration following Na^+ absorption from the gut. Moreover, this observation supports the notions that absorption of Na^+ from food occurs relatively rapidly (Smith et al. 1995) and that regulatory mechanisms promptly control the attendant upsurge in tissue Na^+ concentrations. Consequently, sampling after a substantial period of starvation (e.g., Kamunde et al. 2003) is unlikely to capture these apparently transient changes in tissue Na^+ levels.

Role of the kidney in Na^+ homeostasis during exposure to elevated dietary NaCl and waterborne Cu

The gill is the main organ for regulating Na^+ in freshwater fish, while the kidney contributes 10–30% to whole-body Na^+ balance depending on the prevailing conditions (McDonald and Wood 1981; Wood 1988, 1995). The role of the kidney is likely greater when branchial Na^+ regulating mechanisms are impaired or strained, for example, by exposure to waterborne ionoregulatory toxicants, or when freshwater fish are faced with higher Na^+ loads, for example, via dietary accretion. In the present study, exposure of rainbow trout to elevated waterborne Cu resulted in an initial decline in kidney Na^+ concentrations, which remained depressed in the fish maintained on LS diet. However, in fish maintained on HS diet, renal Na^+ concentrations recovered and surpassed values of the controls and the Cu-unexposed fish maintained on HS diet, suggesting that compensatory renal Na^+ retention occurs in animals faced with branchial Na^+ loss when Na^+ availability via the diet is maintained. These observations are in accord with Grosell et al. (1998) who reported that during acclimation to waterborne Cu, renal Na^+ efflux is reduced owing to increased tubular re-absorption, suggestive of renal

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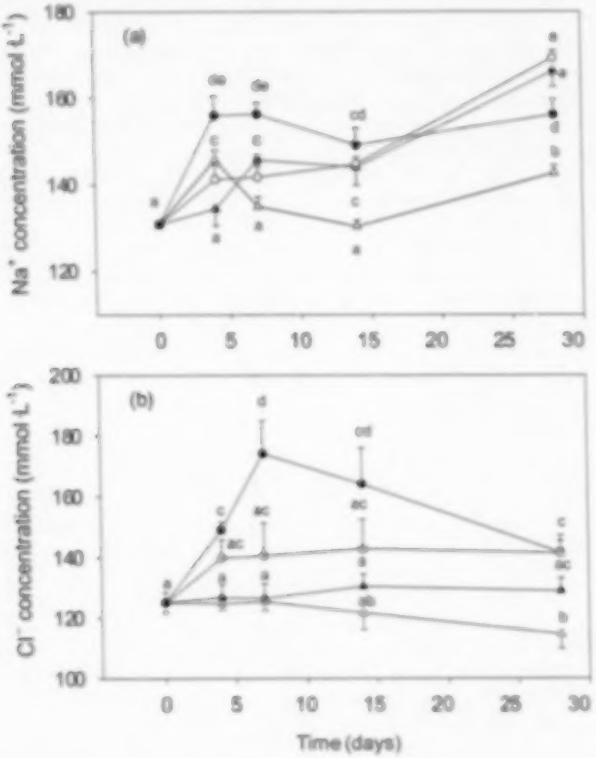
Fig. 6. Effect of elevated dietary NaCl and waterborne Cu exposure on levels of (*a*) plasma Na^+ and (*b*) Cl^-. Elevated dietary NaCl increased both the Na^+ and Cl^- levels, whereas elevated waterborne Cu reduced them. Points on the same panel with different letters are significantly different ($P < 0.05$). ▲, LS diet + no Cu; ●, HS diet + no Cu; △, LS diet + 55 μg·L^{-1} waterborne Cu; ○, HS diet + 55 μg·L^{-1} waterborne Cu.



Na^+ conservation as part of the mechanism of acclimation to waterborne Cu. The lack of Cu accumulation in kidneys of Cu-exposed fish maintained on HS diet likely indicates that the processes involved in renal Na^+ conservation concomitantly impede Cu accumulation. Moreover, although elevated dietary NaCl increases renal Na^+ excretion, in the absence of waterborne Cu exposure the gill remains the main site for Na^+ homeostasis in fish (Salman and Eddy 1987).

Role of the gut in Na^+ homeostasis during exposure to elevated NaCl and waterborne Cu

In addition to digestion and absorption of food, the fish gut has been shown to have an important role in ion regulation (Evans 1979; Smith et al. 1989). Specifically, exposure of freshwater fish to elevated dietary NaCl suppresses branchial Na^+ uptake (Smith et al. 1995; Kamunde et al. 2003; Pyle et al. 2003), increases diffusive Na^+ efflux via the gills (Smith et al. 1995; Pyle et al. 2003), and increases renal Na^+ excretion (Salman and Eddy 1987). Our data are consistent with these findings. Interestingly, little Na^+ is lost through fecal material even in fish fed high Na^+ diets (Salman and Eddy 1988), suggesting that reduction of gastrointestinal Na^+ uptake may not be important in Na^+ homeostasis in freshwater fish. However, during simultaneous exposure to elevated dietary NaCl and waterborne Cu, the gut appears to play a role in conserving Na^+. As with the kidney, gut tissue Na^+ concentration recovered from an initial depression and surpassed values of the controls and the Cu-unexposed fish maintained on HS diet, suggesting that compensatory gut Na^+ absorption occurs in the face of branchial loss when Na^+ availability via the diet is maintained. Because the amount of Na^+ absorbed from food is likely dependent on the animal's Na^+ balance at the time of measurement, future studies need to measure actual uptake rates and fecal losses of Na^+ during exposure to graded levels of both dietary NaCl and waterborne Cu.

Table 1. Effect of exposure of juvenile rainbow trout (*Oncorhynchus mykiss*) to elevated dietary NaCl and waterborne Cu on plasma Na:Cl ratios.

	Group			
Day	LS + no Cu	LS + Cu	HS + no Cu	HS + Cu
0	1.13±0.05	1.13±0.05	1.13±0.05	1.13±0.05
4	1.07±0.04	1.14±0.03	1.05±0.02	1.05±0.05
7	1.16±0.05	1.08±0.03	0.94±0.08*	1.04±0.07
14	1.11±0.05	1.08±0.05	0.94±0.07*	1.04±0.06
28	1.29±0.03	1.25±0.04	1.11±0.04*	1.22±0.05

Note: *, significantly different from LS + no Cu ($P < 0.05$); LS, low salt; HS, high salt.

Plasma Na^+ and Cl^- concentrations

Several studies have demonstrated that elevated waterborne Cu exposure decreases both plasma Na^+ and Cl^- levels in freshwater fish (McKim et al. 1970; Christensen et al. 1972; Wood 2001) and that the Na^+ and Cl^- losses occur in parallel (Stagg and Shuttleworth 1982; Wilson and Taylor 1993; Pelgrom et al. 1995). This indicates that the regulatory mechanisms of Na^+ and Cl^- are, at least in part, linked. In the present study, plasma Na:Cl ratios remain relatively constant in Cu-exposed fish compared with the unexposed fish, indicating that elevated waterborne Cu exposure affected Na^+ and Cl^- in a similar manner and that Na^+ and Cl^- homeostatic mechanisms are coupled. Indeed, in the current model of branchial ion transport in freshwater fish, Na^+ and Cl^- transport are indirectly coupled by carbonic anhydrase (Wood 2001; Grosell et al. 2002). Surprisingly, Na:Cl ratio analysis in the Cu-unexposed fish maintained on HS diet indicated that elevated dietary NaCl exposure increased plasma Cl^- to a greater extent than it did plasma Na^+ concentration, which is generally indicative of the condition of metabolic acidosis reflected in a decrease in plasma HCO_3^- concentration (Wood 2001). Interestingly, metabolic acidosis associated with a decrease in the strong ion difference (SID) between plasma Na^+ and Cl^- concentrations is a well-known effect of exposure to high waterborne NaCl levels (reviewed by Walker et al. 1989). The mechanism of this differential effect of elevated dietary NaCl exposure on the relative plasma concentrations of Na^+ and Cl^- is at present unknown but we speculate a greater inhibition of Na^+ influx and (or) greater stimulation of Na^+ efflux relative to Cl^-. Indeed, some mechanisms of red blood cell (rbc) volume regulation can cause differential transport of Na^+ and Cl^- between the plasma and rbc compartments. For example, exposure of whole blood to

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elevated NaCl causes an initial rbc shrinkage followed by regulatory volume increase (RVI) with increased intracellular Na^+ (Brauner et al. 2002), which can cause preferential decrease in plasma Na^+ relative to Cl^-.

Cu homeostasis in freshwater fish: is plasma Cu concentration the homeostatic set point?

Based on constant plasma Cu concentrations during exposure of fish to elevated waterborne and (or) dietary Cu levels (Grosell et al. 1997, 1998; Kamunde et al. 2001), a Cu homeostatic system geared toward maintaining optimum plasma Cu concentration has been proposed (Kamunde and Wood 2004). Consistent with this proposal, overt toxicity is not observed when plasma concentrations are maintained in face of chronic sublethal Cu exposures (Grosell et al. 1997; Kamunde et al. 2002; Kamunde and Wood 2004). In the present study, we observed chronic toxicity (mortality and growth retardation) concomitant with elevated plasma Cu concentration, demonstrating that elevation of plasma Cu is associated with toxicity, a further indication that plasma Cu concentration is likely the set point in Cu homeostasis. Furthermore, because chronic elevation of plasma Cu was associated with chronic depression of Na^+ and Cl^- concentrations in plasma, whole body, and various tissues, loss of tissue Na^+ and Cl^- is likely a mechanism of chronic waterborne Cu toxicity, akin to acute toxicity, except that the magnitude of the depression is less in chronic toxicity.

In conclusion, elevated dietary NaCl protects juvenile rainbow trout from waterborne Cu-induced mortality, growth, and ionoregulatory impairment and impedes Cu accumulation in target tissues. We provide data suggesting that ionoregulatory disturbance (Na^+ and Cl^- loss) is the mechanism of chronic waterborne Cu toxicity, akin to acute Cu toxicity. Moreover, extrabranchial mechanisms of ion regulation in fish operating at the gut and the kidney appear to play greater roles in maintaining the Na^+ balance during Na^+ loading or Na^+ loss inducing challenges.

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Mercury methylation in the hypolimnetic waters of lakes with and without connection to wetlands in northern Wisconsin

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Abstract: Rates of Hg methylation and demethylation were measured in anoxic hypolimnetic waters of two pristine Wisconsin lakes using stable isotopes of Hg as tracers. One of the lakes is a clear-water seepage lake situated in sandy terrain with minimal wetland influence. The other is a dark-water lake receiving channelized inputs from a relatively large terrestrial wetland. Methyl mercury (MeHg) accumulated in the anoxic hypolimnia of both lakes during summer stratification, reaching concentrations of 0.8 ng·L^{-1} in the clear-water lake and 5 ng·L^{-1} in the dark-water lake. The stable isotopic assays indicated that rate constants of $Hg^{(II)}$ methylation (K_m) ranged from 0.01 to 0.04·day^{-1} in the clear-water lake and from 0.01 to 0.09·day^{-1} in the dark-water lake, depending on the depth stratum. On average, K_m was threefold greater in the dark-water lake. Hypolimnetic demethylation rate constants (K_{dm}) averaged 0.03·day^{-1} in the clear-water lake and 0.05·day^{-1} in the dark-water lake. These methylation rates were sufficient to account for the observed accumulation of MeHg in hypolimnetic water during summer in both lakes. Despite substantial export of MeHg from the wetland to the dark-water lake, our study indicates that in-lake production and decomposition of MeHg dominated the MeHg cycle in both lakes.

Résumé : L'utilisation des isotopes stables de mercure comme traceurs nous a servi à mesurer les taux de méthylation et de déméthylation de Hg dans les eaux anoxiques de l'hyplimnion de deux lacs non perturbés du Wisconsin. Un des lacs est un lac à eau claire alimenté par le ruissellement et situé sur un terrain sablonneux avec peu d'influence des terres humides. L'autre est un lac à eau foncée qui reçoit des apports par canalisations provenant d'une grande région de terres humides terrestres. Le méthylmercure s'accumule dans l'hypolimnion anoxique des deux lacs durant l'été, pour atteindre des concentrations de 0,8 ng·L^{-1} dans le lac à eau claire et 5 ng·L^{-1} dans le lac à eau foncée. Les dosages d'isotopes stables indiquent que les constantes des taux de méthylation de $Hg^{(II)}$ (K_m) varient de 0,01 à 0,04·jour^{-1} dans le lac à eau claire et de 0,01 à 0,09·jour^{-1} dans le lac à eau foncée, en fonction de la profondeur. En moyenne, K_m est trois fois plus élevé dans le lac à eau foncée. Les constantes du taux de déméthylation (K_{dm}) dans l'hypolimnion sont en moyenne de 0,03 jour^{-1} dans le lac à eau claire et de 0,05 jour^{-1} dans le lac à eau foncée. Ces taux de méthylation suffisent à expliquer l'accumulation observée de MeHg dans les eaux de l'hypolimnion au cours de l'été dans les deux lacs. Malgré une importante exportation de MeHg des terres humides vers le lac à eau foncée, notre étude indique que la production et la décomposition de MeHg dans le lac lui-même dominent le cycle de MeHg dans les deux lacs.

[Traduit par la Rédaction]

Introduction

For decades, elevated levels of Hg in aquatic systems have been responsible for health advisories on fish consumption in many countries (US Environmental Protection Agency 1997). Pristine lakes receive Hg inputs through long-range atmospheric transport from industrial sources (Trip and Allan 2000). The majority of Hg entering lakes from the atmosphere is in the inorganic form; however, essentially all of the Hg accumulating in fish is methyl mercury (MeHg) (Bloom 1992). Understanding all of the sources of MeHg to aquatic systems is imperative for the management of Hg pollution.

There are three main sources of MeHg to remote lakes: direct precipitation, watershed runoff (especially from wet-

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lands), and the in-lake methylation of inorganic Hg (Rudd 1995). The relative importance of these sources reportedly varies with the rates of MeHg deposition from the atmosphere, lake type, and catchment hydrology (Rudd 1995). In-lake methylation is known to occur in both littoral sediments (e.g., Gilmour and Riedel 1995) and the anoxic water column (Watras et al. 1995*a*; Mauro et al. 2002). Historically, there has been an emphasis on methylation in the sediments (Korthals and Winfrey 1987; Ullrich et al. 2001) and inputs from the watershed (St. Louis et al. 1994; Lee et al. 1998) as important sources of MeHg to lakes. Several mechanisms have been proposed to explain the seasonal accumulation of MeHg in the anoxic hypolimnia of dimictic lakes including (*i*) diffusion from profundal sediments to the water column under anoxic conditions (Gagnon et al. 1997; Gill et al. 1999; Ullrich et al. 2001), (*ii*) methylation in the anoxic water column (Henry et al. 1995; Watras et al. 1995*a*; Mauro et al. 2002), and (*iii*) sedimentation of catchment derived particulate MeHg and methylation in the anoxic water column (Verta and Matilainen 1995).

Although Hultberg et al. (1994) found that inputs of MeHg from the watershed could account for all of the MeHg accumulated by fish in a Swedish Lake, and although wetlands have been implicated as important sources of MeHg to receiving waters (St. Louis et al. 1994; Hurley et al. 1995; Lee et al. 1998), the relative importance of internal and external inputs of MeHg remains unclear — perhaps varying widely with hydrologic conditions (Sellers et al. 2001). Here, we investigate the internal production of MeHg in two Wisconsin lakes from very different hydrologic settings. One is a clear-water, precipitation-dominated seepage lake in a relatively small, sandy terrestrial catchment. Previous studies indicate that direct atmospheric deposition is the major source of $Hg^{(II)}$ and that internal production is the major source of MeHg for this lake, although the relative importance of littoral versus profundal methylation remains in question (Watras et al. 1995*a*, 1995*b*; Krabbenhoft et al. 1998). The other is a dark-water drainage lake that receives most of its water via channelized runoff from a relatively large wetland.

This study uses a new analytical tool (stable isotopes of Hg) to build on the findings of previous research on water column methylation in Wisconsin lakes (Watras et al. 1995*a*; Mauro et al. 2002). The present study uses short incubation times under ambient conditions with low-level spikes of stable $Hg^{(II)}$ and a more rigorous time series (six time points in 25 h) to better quantify rates during the critical early stages of the incubations. We also monitored changes in water chemistry and microbial community structure during the incubations to assess the preservation of in situ lake conditions. A first attempt was made to quantify demethylation rate constants (K_{dm}) in these lakes, since K_{dm} is expected to play a critical role in determining net MeHg production and accumulation (Hintelmann et al. 2000; Sellers et al. 2001).

Materials and methods

Site descriptions

Pallette Lake (45°59′N, 89°42′W) and Devils Lake (45°31′N, 88°52′W) are both mesotrophic lakes located in northern Wisconsin, USA. Pallette Lake is a clear seepage lake with a surface area of 70 ha and a maximum depth of 18.3 m. Anoxia develops at approximately 14 m by mid-August in this lake and the stratification of microbial communities above and below the oxic–anoxic boundary is striking (Watras and Bloom 1994). Devils Lake is a dystrophic drainage lake with a surface area of 12.5 ha and a maximum depth of 7 m. Anoxia develops at around 3.5 m by mid-August. The inlet stream to Devils Lake emanates from a 24-ha wetland roughly 0.75 km from the lake. Stream water flowing from the wetland has high concentrations of dissolved organic C (DOC) (30–85 mg C·L^{-1}), total Hg (HgT) (7–16 ng·L^{-1}), and total MeHg (MeHgT) (0.2–0.8 ng·L^{-1}) and low pH (3.5–4.5) (C.J. Watras et al., unpublished data). While both lakes are in remote locations in undeveloped watersheds, Devils Lake has a notably higher (roughly four times) MeHg concentration than Pallette Lake (0.2–0.4 versus 0.03–0.09 ng·L^{-1}, surface).

Field sampling

In August 2002, Devils Lake and Pallette Lake were sampled using a vertical profiling system (Seabird 19 plus conductivity–temperature–depth (CTD) instrument) that measures several parameters such as dissolved oxygen (DO), beam attenuation, chlorophyll fluorescence, and pH throughout the water column at a frequency of 4 Hz. The fine-scale bio-optical profiles were used to target discrete depths for sampling Hg species and other analytes. Clean techniques were followed throughout all phases of sample collection, transport, storage, and analysis (US Environmental Protection Agency 1996). Water samples were collected from discrete depths using an all-plastic submersible pump attached to an acrylic outrigger that was fixed to the Seabird CTD. The inlet of the pump was aligned with the CTD sensors and the outlet was attached to 1.3-cm internal diameter C-Flex™ tubing that ran to the surface. An in-line Nitex screen (64 µm) was used to remove zooplankton.

Samples for ambient Hg analyses were collected in 2-L acid-cleaned glass bottles that were rinsed with lake water three times to condition the bottle walls. Water for methylation and demethylation assays was collected in acid-cleaned 160-mL darkened glass serum bottles that avoided exposure to sunlight. To preserve in situ redox conditions, the incubation bottles were overfilled thrice their volume and capped by displacing air and water through a needle injected into the rubber stopper. Samples were double ziplock bagged for transport in a cooler back to the laboratory. The cooler was monitored with a thermometer and approximate in situ conditions were maintained during transportation (less than 2 h from collection).

At the Trout Lake Station Laboratory, 460 pg of inorganic ^{199}Hg (92% pure (Trace Sciences International), stock stored in 0.2% HCl and diluted with Milli-Q water to yield the working standard less than 2 weeks before sampling) and 350 pg of $Me^{201}Hg$ (98% pure (Trace Sciences International), synthesized from ^{201}HgO using methylcobalamin, stored in 50/50 (*v*/*v*) isopropanol–water and 0.2% HCl, and diluted with Milli-Q water to yield the working standard less than 2 weeks before sampling) were added to each sample through the ziplock bags via an analytical syringe (Hamilton

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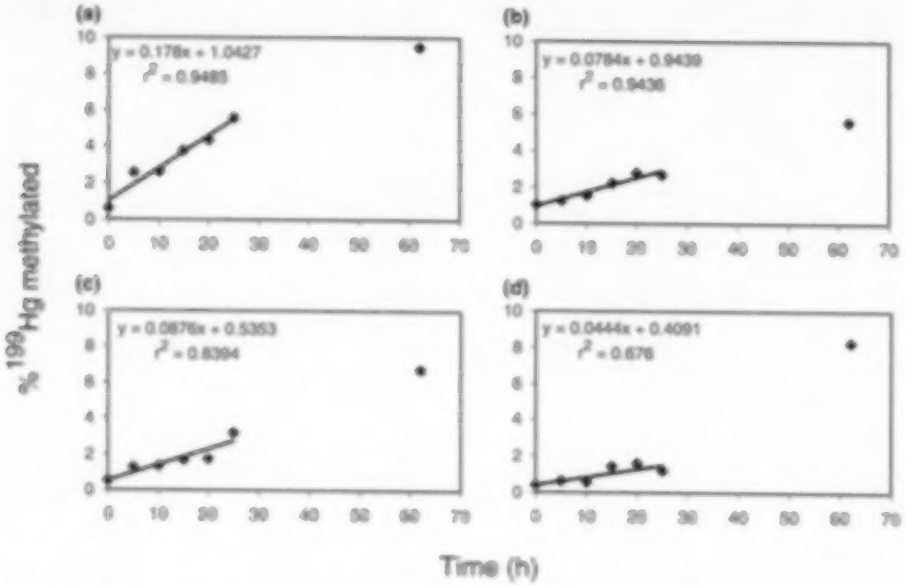
Fig. 1. Methylation time series and regression analysis used to estimate K_m at four hypolimnetic depths in Pallette Lake: (*a*) 14.0 m, (*b*) 15.0 m, (*c*) 16.0 m, and (*d*) 17.5 m.



Gastight No. 84880, 25 µL) that punctured the resealable rubber stopper. In Devils Lake, the inorganic ^{199}Hg spike increased the HgT concentration between 30% and 50% and the Me^{201}Hg spike increased the MeHgT concentration between 40% and 125%. In Pallette Lake, the inorganic ^{199}Hg spike increased the HgT concentration between 200% and 300% and the MeHgT was increased between 300% and 750%. The increase in isotopic Hg in Pallette Lake may appear large, but the isotopic concentrations of 2.9 ng inorganic ^{199}Hg·L^{-1} and 2.2 ng Me^{201}Hg·L^{-1} were less than the ambient levels in Devils Lake. Samples were incubated using two refrigerators set to the in situ lake temperatures. Incubations were terminated by addition of 0.5 mL of concentrated HCl at 5-h intervals for the first 25 h to obtain methylation and demethylation rates. An additional sample at each depth was incubated for 48 h at Devils Lake and 62 h at Pallette Lake. The time zero sample was obtained by adding HCl before the isotopes.

MeHg determination

Analysis of ambient Hg species was performed at the Trout Lake Station Laboratory and followed Morrison and Watras (1999). The methylation and demethylation samples were analyzed at Trent University, Ontario, following Hintelmann and Ogrinc (2003). The formation and degradation of MeHg were determined by monitoring the concentrations of the respective isotopes Me^{199}Hg and Me^{201}Hg. MeHg was distilled from the incubation samples, which was followed by ethylation, purging with nitrogen gas, trapping on Tenax®, thermal desorption, separation by gas chromatography, and detection with an inductively coupled plasma mass spectrometer. Me^{202}Hg (99% pure (Trace Sciences International)) was added as an internal standard to correct for procedural recoveries. Chromatographic data were collected from the inductively coupled plasma mass spectrometer and peak areas were used to calculate concentrations using a programmed spreadsheet that accounts for procedural blanks and the purities of the isotopes (as described in Hintelmann and Ogrinc 2003).

Methylation and demethylation rate constants

Following Hintelmann et al. (2000), we used eq. 1 below to calculate the methylation rate constant:

$$(1) \qquad [Me^{199}Hg_t] = [Me^{199}Hg_{t-1}] + K_m[^{199}Hg_t^{(II)}]$$

where t represents incubation time (h). The methylation rate at each depth was obtained from the slope of the regression line $[Me^{199}Hg]$ versus time fitted to the points for the first 25 h of the incubation (Figs. 1 and 2). The methylation rate constant (K_m) was obtained assuming that $[^{199}Hg]$ remained constant during this time. Considering the negligible consumption of $[^{199}Hg]$ by methylation (the maximum was <10%), this approximation seemed justified, as indicated by the linearity of $[Me^{199}Hg]$ versus time. The methylation potential is expressed as the percent Me^{199}Hg formed from the inorganic ^{199}Hg spike (%·day^{-1}) or as the methylation rate constant K_m (day^{-1}). To determine differences between K_m values at different depths, an analysis of covariance test for parallelism (using the F test) was performed using Statistica™. The methylation data from Pallette Lake met the parametric assumptions of the analysis of covariance test — the residuals showed homoscedasticity (Levene test, $p > 0.05$) and a normal distribution (Shapiro–Wilk's test, $p > 0.05$), and there was a linear trend between the response variable and covariate (Fig. 1). At Devils Lake, there was a linear trend between the response variable and the covariate (Fig. 2), but the residuals from two of the five sample depths were not homoscedastic (Levene test, $p < 0.05$; however, by graphical assessment, no trend was visually discernable), nor

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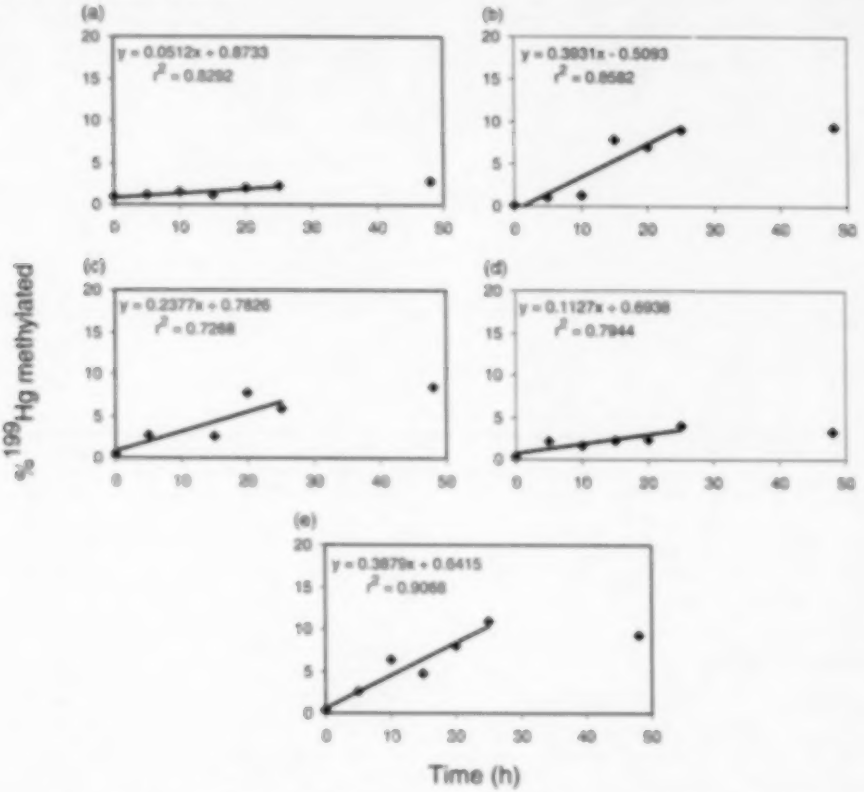
Fig. 2. Methylation time series and regression analysis used to estimate K_m at five hypolimnetic depths in Devils Lake: (*a*) 3.0 m, (*b*) 4.5 m, (*c*) 4.9 m, (*d*) 5.5 m, and (*e*) 6.0 m.



were the residuals normally distributed (Shaprio–Wilk's test, $p < 0.0001$). Lindman (1974) noted that the F test is remarkably robust to deviations from normality, indicating that it is still appropriate to use this test without transforming the Devils Lake data. For both lakes, the range and average of the Durbin–Watson test statistic for the individual regression time series suggested that significant autocorrelation was not likely to exist because the values were close to 2 (overall: $d = 2.0 \pm 0.4$; Devils Lake: $d = 2.2 \pm 0.4$; Pallette Lake $d = 1.8 \pm 0.3$). The detection limit for the formation of $Me^{199}Hg$ (Hintelmann and Evans 1997) is affected by the ambient MeHg concentration in the lakes and was 0.011 ng·L^{-1} at Devils Lake and 0.002 ng·L^{-1} at Pallette Lake (corresponding to 0.40% and 0.07% of the added $^{199}Hg^{(II)}$ methylated, respectively).

The demethylation rate constant K_{dm} was obtained from the linear regression of ln[$Me^{201}Hg$] versus time (Hintelmann et al. 2000). Based on the variability of the time = 0 samples, the precision associated with the $Me^{201}Hg$ isotope additions was determined to be 11% (mostly attributed to variability associated with the analytical syringe). None of the demethylation assays had demethylation occurring above the 11% variability. A K_{dm} value of 0.12·day^{-1} (based on a maximum 11% loss of $Me^{201}Hg$ over time) gives an upper limit to demethylation activity. Since we were unable to quantify K_{dm} at specific depths with reasonable certainty, we pooled the available data and regressed ln[$Me^{201}Hg$] versus time for all depths to obtain a global estimate of K_{dm} in the hypolimnion of each lake. The 90% confidence intervals bounding the slopes of the two regressions add additional constraints to the probable range of K_{dm} for a given depth and lake.

Ancillary water chemistry

Samples for water chemistry analysis were collected in 4-L plastic carboys, except for DOC and sulfide. The DOC samples were filtered through precombusted 0.4-µm glass fiber filters (using a precleaned all-glass syringe) into precombusted glass vials with Teflon-lined screw caps. Sulfide samples were collected into 60-mL serum bottles using the overflow technique to exclude oxygen. The serum bottles were sealed with Teflon septa. Dissolved sulfide (detection limit 0.1 µmol·L^{-1}) was determined using an Orion model 94-16BN silver/sulfide electrode with an Orion double-junction reference as described by van Gemerden (1987). Analysis for other constituents followed Watras et al. (1995*b*).

Microbial community analysis

To assess potential biological changes during the in vitro incubations, we estimated bacterial community composition at the beginning and end of each experiment by automated ribosomal intergenic spacer analysis (ARISA) following a modification of the method of Fisher and Triplett (1999). Similarity of the bacterial community profiles before and after incubation of the samples was analyzed using the

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Table 1. Mercury, MeHg, and ancillary constituents in the water column of Pallette Lake and Devils Lake.

Lake	Depth (m)	HgT (ng·L^{-1})	MeHgT (ng·L^{-1})	DOC (mg·L^{-1})	SPM (mg·L^{-1})	pH	ANC (μequiv·L^{-1})	Specific conductivity (μS·cm^{-1})	Total P (μg·L^{-1})	NO_3-N (mg·L^{-1})	SO_4 (mg·L^{-1})
Pallette	3.0	0.38	bd	5.05	2.17	6.9	108	18.8	7	<0.006	1.82
Pallette	6.5	0.49	bd	5.36	4.89	9.4	109	28.0	13	<0.006	1.90
Pallette	8.5	0.42	bd	5.31	6.43	9.3	110	32.6	19	<0.006	1.94
Pallette	9.8	0.38	bd	4.71	4.07	6.4	111	20.0	21	0.082	1.99
Pallette	14.0	0.97	0.22	4.60	5.24	6.9	142	23.6	49	<0.006	2.12
Pallette	15.0	1.40	0.62	4.85	6.21	6.2	185	29.4	81	<0.006	1.80
Pallette	16.0	1.42	0.72	4.91	6.43	6.3	193	28.3	101	<0.006	1.58
Pallette	17.5	1.43	0.76	4.91	6.67	6.3	207	32.4	109	0.021	1.52
Devils	1.5	2.25	0.34	10.75	1.17	5.6	84.6	19.1	13	<0.006	2.80
Devils	3.0	5.02	1.72	11.04	5.34	5.5	28.3	16.9	22	<0.006	2.98
Devils	4.5	7.40	3.36	12.15	4.94	5.7	114	25.5	32	<0.006	2.29
Devils	4.9	7.08	3.55	12.28	4.01	5.8	34.4	27.8	37	<0.006	2.10
Devils	5.5	8.32	4.85	12.95	3.60	6.0	63.7	27.2	75	<0.006	1.51
Devils	6.0	9.49	5.27	13.56	4.95	6.1	69.1	32.2	116	<0.006	0.86

Note: The water column was not sampled at a sufficiently fine spatial scale to reveal maxima of Hg or MeHg in the hypolimnion, as previously regions of the anoxic–sulfidic water column. The DOC in Pallette Lake is not chromophoric dissolved organic matter (CDOM) of terrestrial origin; Lake DOC is predominantly CDOM originating from a wetland in the terrestrial watershed. SPM, suspended particulate matter; ANC, acid-neutralizing

Sorenson's index of similarity (Magurran 1988). Calculation of Sorenson's indices was carried out using the PRIMER 5 software package (PRIMER-E, Plymouth, UK).

Results and discussion

Vertical profiles of Hg species and other analytes

In Pallette Lake, HgT and MeHgT concentrations both increased in the hypolimnion (Table 1; Fig. 3). The percentage of MeHgT to HgT increased from less than 12.1 ± 1.4% (MeHgT was below the detection limit of 0.05 ng·L^{-1}) in the epilimnion to 49.4 ± 4.6% in the anoxic hypolimnion. Dissolved MeHg ($MeHg_d$) in the epilimnion (above 8.5 m) was below the detection limit of 0.05 ng·L^{-1} and particle-associated MeHg ($MeHg_p$) ranged from 0.01 to 0.04 ng·L^{-1}. At 17.5 m, almost all of the MeHg was dissolved. In the hypolimnion, the amount of HgT_p that was $MeHg_p$ was 25 ± 15%, while the percentage of HgT_d that was $MeHg_d$ was 75 ± 14%, potentially reflecting lower particle reactivity and (or) higher solubility of MeHg than of $Hg^{(II)}$ during sulfidic conditions (Gagnon et al. 1997) or reflecting depletion of dissolved $Hg^{(II)}$ by microbial cells and subsequent release of Hg as MeHg (Benoit et al. 2001).

In Devils Lake, the MeHg concentrations were roughly four times higher than in Pallette Lake. As in Pallette Lake, the concentration of HgT and MeHgT both increased with depth (Table 1; Fig. 3). In the epilimnion, the percentage of MeHgT to HgT was 15%, which increased to 52 ± 6% in the anoxic hypolimnion. The amount of Hgd that was MeHg was 13% in the epilimnion and increased to 65 ± 8% in the anoxic hypolimnion. The percentage of $MeHg_p$ to HgT_p was 17 ± 10% with no visible trend with depth. Almost all of the MeHg in the lake was in the dissolved form, with $MeHg_p$ <10% throughout the water column, except at 1.5 m where $MeHg_p$ was slightly higher.

The pH of hypolimnetic water in Devils Lake and Pallette Lake was similar, ranging from 6.1 to 6.3, which is characteristic of sulfate-reducing environments (Morel 1983). Specific conductivity and concentrations of major nutrients, anions, and cations were also similar in both lakes, with the exception of potassium, iron, and aluminum, which were higher in Devils Lake. Devils Lake also had slightly higher sulfate concentrations in the epilimnion and slightly lower sulfate concentrations in the hypolimnion compared with Pallette Lake. Consistent with the larger decline in sulfate, there was also a larger buildup of sulfide in the anoxic waters of Devils Lake than in Pallette Lake. The DOC concentration at Devils Lake was roughly twofold higher than at Pallette Lake, but this difference underestimates the relative importance of dissolved allochthonous humic matter in Devils Lake. As evidenced by the high water clarity and the spectral distribution of downwelling light (Watras and Baker 1988), most of the DOC in Pallette Lake comprises autochthonous plankton exudates of low color. In Devils Lake, the deep brown stain and low transparency indicate high concentrations of humic matter of wetland origin.

Possible explanations for the elevated levels of MeHg in the two hypolimnia include diffusion of MeHg from the profundal sediments, downward transport of MeHg by settling particles, and methylation occurring within the anoxic water column. A possible explanation for the higher MeHg concentrations in Devils Lake than in Pallette Lake is inputs of MeHg from the wetland affecting Devils Lake. Another possibility is that humic matter, $Hg^{(II)}$, and sulfate received from the wetland stimulated the production of MeHg and increased the stability and retention of MeHg in the water column of Devils Lake. For both lakes, we used the results from the Hg methylation and demethylation assays to assess if MeHg production in the water column could explain the observed buildup of MeHg during summer stratification (see below).

In vitro incubation conditions

ARISA profiles generated from the microbial communities present in the incubation bottles at the initial time point and after 25 h indicated that there was no significant change

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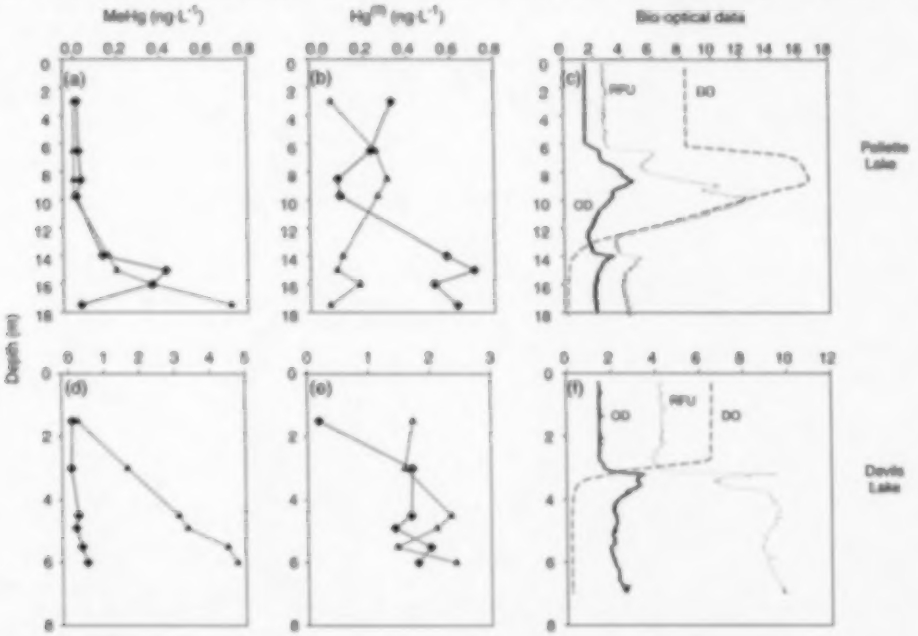
H_2S ($mg \cdot L^{-1}$)	Cl ($mg \cdot L^{-1}$)	Ca ($mg \cdot L^{-1}$)	Mg ($mg \cdot L^{-1}$)	Total K ($mg \cdot L^{-1}$)	Na ($mg \cdot L^{-1}$)	Fe ($\mu g \cdot L^{-1}$)	Mn ($\mu g \cdot L^{-1}$)	Total Al ($\mu g \cdot L^{-1}$)
na	0.22	2.0	0.57	0.34	0.47	11	1.6	<17
na	0.22	2.0	0.57	0.33	0.46	18	3.2	<17
na	0.34	2.1	0.61	0.38	0.40	21	4.5	<17
na	0.24	2.1	0.59	0.39	0.44	35	7.6	<17
0.03	0.22	2.0	0.60	0.43	0.48	260	48	<17
0.20	0.22	2.4	0.60	0.44	0.47	660	63	19
0.45	0.24	2.5	0.60	0.46	0.47	800	70	<17
0.50	0.33	2.5	0.60	0.45	0.45	870	73	<17
0.01	0.18	1.5	0.48	0.62	0.25	360	47	110
0.01	0.22	1.9	0.49	0.80	0.27	970	71	150
0.19	0.23	2.1	0.51	0.85	0.30	1800	71	180
0.31	0.23	2.1	0.51	0.86	0.66	1900	71	190
0.72	0.24	2.2	0.53	0.86	0.28	2300	75	210
1.03	0.24	2.4	0.55	0.88	0.32	2800	78	240

observed in other Wisconsin lakes. The objective here was simply to measure concentrations and rates in various rather, it is colorless dissolved organic matter (DOM), likely derived from plankton exudates. In contrast, Devils capacity; na, not available; bd, below detection limit.

Fig. 3. Depth distribution of waterborne Hg species (triangles, dissolved; diamonds, particulate) and selected lake parameters. (*a* and *d*) MeHg; (*b* and *e*) $Hg^{(II)}$; (*c* and *f*) DO ($mg \cdot L^{-1}$), chlorophyll fluorescence (RFU), and beam attenuation (OD) (m^{-1}). Note that concentrations of Hg species that were below detection in the upper water of Pallette Lake are plotted here at the detection limit.



in the composition of the microbial community at any of the depths sampled (Fig. 4). The magnitude of the ARISA peaks does not exactly reflect the abundance of the bacterial population in the original sample due to preferential amplification of some templates during polymerase chain reaction amplification. For this reason, we chose to evaluate only the presence or absence of peaks in comparing ARISA profiles. However, similar results are obtained when peak area is considered using the Bray–Curtis similarity index. Major anions (sulfate, fluoride, nitrate, and phosphate), cations (lithium, sodium, ammonium, potassium, calcium, and magnesium), and DOC were monitored at each incubation time interval and were shown to remain constant through the duration of the experiment (Fig. 5 shows data for sulfate). These data indicate that in situ conditions were maintained during the incubation assays and offer reassurance that the methylation

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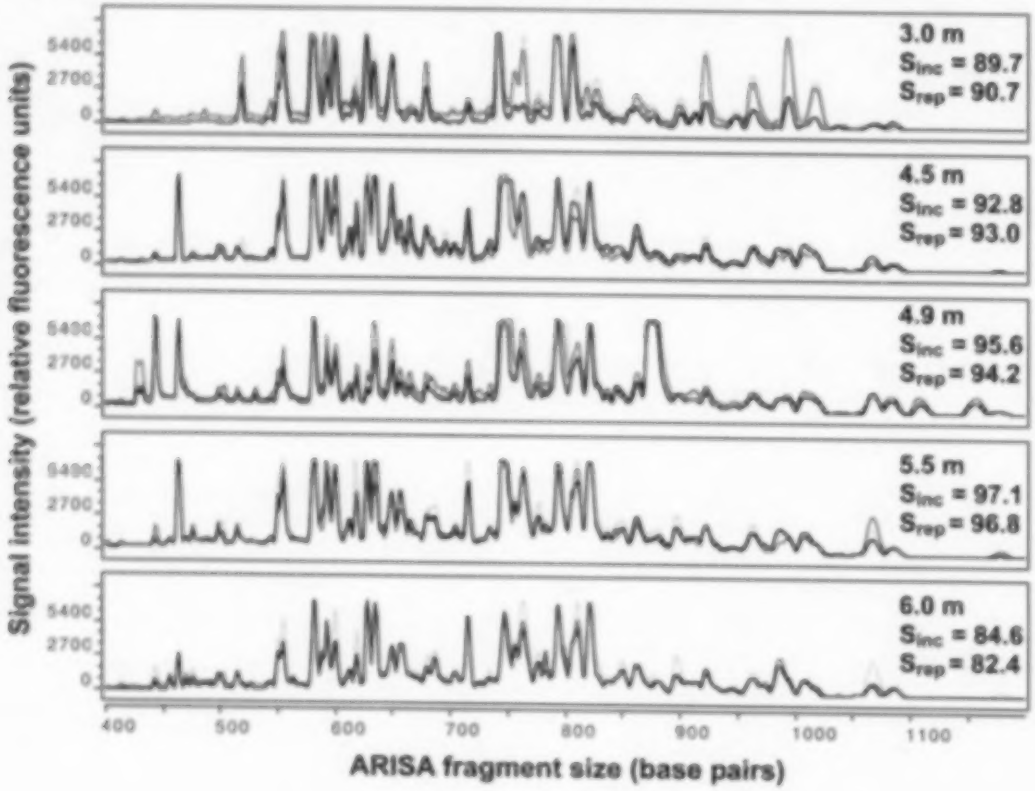
Fig. 4. ARISA profiles generated from microbial communities before (solid line) and after (shaded line) incubation for 25 h in Devils Lake. Sample depth and average Sorenson's index (S_{inc}) for comparisons between the initial time point and the replicate profiles from the incubated samples are indicated on each panel along with the Sorenson's index for comparisons between the replicate profiles (S_{rep}). The Sorenson's index of similarity indicates the percentage of peaks in common between samples. Variability between ARISA profiles from different time points does not exceed the variation between replicate profiles.



data accurately reflect the in situ methylation activity. However, there is still some uncertainty concerning the bioavailability of the added Hg and the rates presented are perhaps best considered as the in situ methylation potential (Hintelmann et al. 2000).

Water column methylation and demethylation assays

The depths sampled at Pallette Lake for Hg methylation and demethylation assays were 8.5, 13.0, 14.0, 15.0, 16.0, and 17.5 m. These depths were chosen because they coincided with peaks in chlorophyll fluorescence and beam attenuation. No methylation activity was detected at 8.5 and 13.0 m. The water at 8.5 m was above the oxycline and 13.0 m was right at the zone of rapid oxygen depletion.

Methylation activity was detected at 14.0, 15.0, 16.0, and 17.5 m in Pallette Lake (Fig. 1). A peak in methylation activity (4.3%·day^{-1}) was detected at 14.0 m ($F_{[3,10]} = 11.65$, $p = 0.0003$). The peak coincided with the appearance of sulfide (0.9 μmol·L^{-1}) in the water column, which continued to increase with depth (14.6 μmol·L^{-1} at 17.5 m). Also, at the depth of peak methylation activity (14.0 m), MeHgT increased by almost one order of magnitude compared with the 10.0-m stratum (the closest overlying depth at which ambient MeHgT was measured). The lower methylation potentials at 15.0, 16.0, and 17.5 m were statistically indistinguishable and averaged 1.7 ± 0.5%·day^{-1} ($F_{[2,12]} = 2.23$, $p = 0.150$). The MeHgT concentration approximately doubled below 14.0 m (Table 1), seemingly contradicting the measured methylation rates at these depths. However, as discussed below, a possible explanation for the increasing buildup of MeHg with increasing depth below 14.0 m is that methylation started and peaked earlier at the lower depths. The sediment could be an additional source of MeHg, although our modeling work suggests that a sediment source would not be necessary to explain the observed MeHg levels in the hypolimnion (see following section).

At Devils Lake, the depths chosen for methylation and demethylation assays were: 3.0, 4.5, 4.9, 5.5, and 6.0 m. These depths were chosen because they corresponded to peaks in chlorophyll fluorescence, beam attenuation, and anoxic water. Methylation activity was detected at all of the depths sampled (Fig. 2). The lowest methylation potential was at 3.0 m, which corresponded to the transition zone between oxic and anoxic conditions (DO = 3.53 mg·L^{-1}). Two distinct peaks in methylation activity were detected at 4.5 and 6.0 m ($F_{[4,10]} = 7.02$, $p = 0.001$). The two peaks were of very similar size (4.5 m: 9.4%·day^{-1}; 6.0 m: 9.3%·day^{-1}) compared with the potentials detected at the other sample

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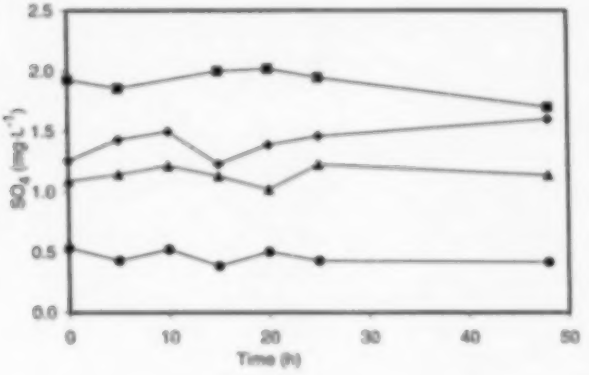
Fig. 5. Stability of sulfate concentrations in incubation bottles during in vitro methylation and demethylation assays in Devils Lake. Squares, 3.0 m; diamonds, 4.5 m; triangles, 4.9 m; circles, 5.5 m.

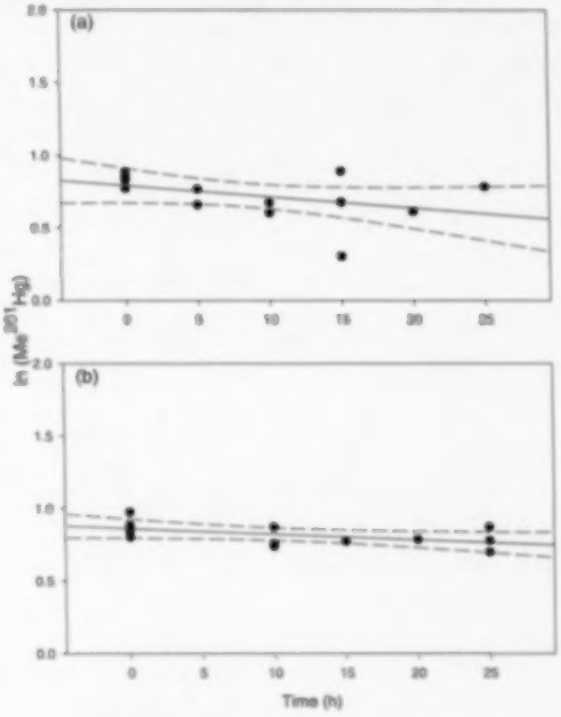


depths (1.2–5.6%·day^{-1}). The peak at 4.5 m corresponded to an increase in sulfide by over an order of magnitude (0.4–5.7 µmol·L^{-1}) and a doubling in the MeHgT concentration compared with 3.0 m. Following the peak at 4.5 m, the methylation potential decreased at 4.9 and 5.5 m before the second peak was detected at 6.0 m. Below 4.5 m, the MeHgT concentration continued to increase between depths sampled but in smaller increments. The concentration of sulfide increased with depth, reaching 30 µmol·L^{-1} at 6.0 m.

The presence of a second peak in methylation potential lower in the water column of equal magnitude to the one below the oxycline has not been observed at other lakes studied (e.g., C.S. Eckley and H. Hintelmann, unpublished data; Watras et al. 1995*a*; Mauro et al. 2002). The DOC content in Devils Lake was highest at 6.0 m (13.6 mg·L^{-1}), which, combined with the presence of sulfate at this depth, may have stimulated the activity of some bacteria, while other bacteria were more favored by the higher sulfate concentrations made available farther up in the water column as the oxycline migrated upwards. It is possible that this second peak is unique to shallow lakes containing high nutrient, DOC, and sulfate concentrations under anoxic conditions. Alternatively, as peaks in methylation activity occur at discrete depths (0.5 m or less thick), it is possible that other studies may have missed this second peak in methylation closer to the sediment, especially since more attention has been paid to sampling the transition zone between oxic and anoxic conditions (C.S. Eckley and H. Hintelmann, unpublished data; Watras et al. 1995*a*). We are presently investigating possible relationships between methylation rates and the presence of various bacteria (O. Regnell et al., unpublished data).

In the demethylation assays, the slope of the regressions of ln[$Me^{201}Hg$] versus time were negative for both Pallette Lake and Devils Lake, suggesting that demethylation did take place in both hypolimnia, albeit occurring at highly uncertain rates (Fig. 6). Roughly estimated this way, the 90% confidence interval for K_{dm} was 0.001–0.37·day^{-1} in Devils Lake and 0.001–0.18·day^{-1} in Pallette Lake. The role of demethylation is further discussed in the source assessment below.

Fig. 6. Demethylation time series for Devils Lake and Pallette Lake pooling available data for all depths in each lake. Regression analysis indicates that slopes are significantly different from zero at a probability level of 0.13 ((*a*) Devils Lake) and 0.09 ((*b*) Pallette Lake). The 90% confidence interval (broken lines) bounding K_{dm} for Devils Lake extended from 0.02 to –0.39·day^{-1}; for Pallette Lake, it extended from 0.00 to –0.18·day^{-1}.



K_m and the buildup of hypolimnetic MeHg

A simple iterative model was used to test whether the experimentally measured rates of water column methylation were sufficient to account for the accumulation of MeHg in the hypolimnion during summer. The modeling was not designed to take into consideration all of the sources of MeHg but simply to put the measured K_m values in perspective, thus relying on several simplifications to meet our objective.

Iteration, using daily time steps, started with the measured ambient concentration of MeHg during spring assuming well-mixed conditions in both lakes (Table 2) and ended with those measured in the fall of 2002 (mid-September) (Table 3) representing a period of 90 days. To match the observed accumulation of Hg at each depth, the model included an incremental (daily) addition of Hg. This daily addition was estimated for each depth as

$$(2) \qquad Hg_{input} = (Hg_{end} - Hg_{start})/90$$

The source of the "new" Hg input could be from settling epilimnetic particles (rainfall or streamflow) and (or) diffusion from the bottom sediments. The speciation of the Hg_{input} was assumed to be the same as that in the epilimnetic waterborne pool (i.e., 88% $Hg^{(II)}$ and 12% MeHg in Devils Lake based on the mean of five monthly epilimnetic samples

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Table 2. Data indicating that the water columns of both lakes were mixed during the spring.

Lake	Date	Depth (m)	HgT (ng·L^{-1})	MeHgT (ng·L^{-1})	DO (mg·L^{-1})	Temperature (°C)
Devils	30 May 2002	1.1	3.95	0.28	8.6	19.1
Devils	30 May 2002	2.0	4.03	0.20	9.3	14.4
Devils	30 May 2002	5.0	4.25	0.32	5.6	8.0
Pallette	21 March 1990	2.0	0.67	0.067	9.83	4.8
Pallette	21 March 1990	10.0	1.36	0.175	3.38	4.0
Pallette	21 March 1990	18.0	2.54	1.006	0.00	4.5
Pallette	26 April 1990	0.3	0.43	0.040	9.95	14.4
Pallette	26 April 1990	2.0	0.47	0.030	9.8	14.0
Pallette	26 April 1990	6.0	1.11	0.060	9.55	8.4
Pallette	26 April 1990	8.0	0.61	0.030	7.81	7.0
Pallette	26 April 1990	10.0	0.69	0.160	7.46	6.1
Pallette	26 April 1990	12.0	0.47	0.080	6.77	5.8
Pallette	26 April 1990	14.0	0.67	0.080	7.21	5.5
Pallette	26 April 1990	16.0	0.82	na	6.12	5.4

Note: Devils Lake was measured during turnover, whereas Pallette Lake was measured before and after turnover (as indicated by the large decrease in HgT and MeHgT below a depth of 10 m). The HgT and MeHgT values measured in April and May were used to calculate initial concentrations in the modeling work. na, not available.

collected between May and September 2002 and 90% $Hg^{(II)}$ and 10% MeHg in Pallette Lake based on 41 epilimnetic samples (≤12.0 m) from eight sample dates between April and September 1998–2002). These values were then entered into eqs. 3 and 4, which were used to calculate the daily changes in Hg species for each depth in the absence of any demethylation (i.e., $K_{dm} = 0$).

$$(3) \quad Hg_t^{(II)} = Hg_{t-1}^{(II)} + Hg_{input}^{(II)} - K_m Hg_{t-1}^{(II)}$$

$$(4) \quad MeHg_t = MeHg_{t-1} + MeHg_{input} + K_m Hg_{t-1}^{(II)}$$

where t represents successive days of the 90-day iteration. Iteration over the 90-day period showed that without any demethylation, the measured methylation rates (K_m) overestimated the accumulation of MeHg by 207% in Pallette Lake and by 177% in Devils Lake. This overestimation of MeHg indicates the importance of demethylation in lake water and the need to incorporate a demethylation constant (K_{dm}), in the model. We calculated the values of K_{dm} needed to match the measured ambient concentrations at each depth after 90 days using eqs. 5 and 6:

$$(5) \quad MeHg_t = K_m Hg_{t-1}^{(II)} + MeHg_{input} + MeHg_{t-1} e^{-K_{dm}}$$

$$(6) \quad Hg_t^{(II)} = Hg_{t-1}^{(II)} - K_m Hg_{t-1}^{(II)} + MeHg_{t-1}(1 - e^{-K_{dm}}) + Hg_{input}^{(II)}$$

The K_{dm} values calculated from this procedure averaged 0.034·day^{-1} in the hypolimnion of Pallette Lake and 0.055·day^{-1} in Devils Lake (Table 3). The K_{dm} values were also measured directly using stable isotopes; however, owing to difficulties in detecting the small decreases in the added isotope over 25-h incubations, they have an associated 90% confidence interval ranging from 0.02 to 0.39·day^{-1} in Devils Lake and from 0.001 to 0.18·day^{-1} in Pallette Lake (Fig. 6). The utility of using eqs. 5 and 6 to calculate K_{dm} is that it provides a more precise measure of demethylation fitted to the measured ambient MeHg concentrations at each depth. At all depths in both lakes, the calculated K_{dm} values fell within the 90% confidence interval of the measured demethylation constants. These values for K_{dm} yielded half-lives for hypolimnetic MeHg ranging from 1 to 8 weeks. These results indicated that in situ methylation and demethylation could account for the hypolimnetic accumulation of MeHg at each depth in both lakes during summer. In general, K_{dm} varied directly with K_m, a result that is consistent with the hypothesis that methylation and demethylation in the low-light environment of anoxic hypolimnia are both biologically driven, perhaps by the same microbes (e.g., Marvin-Dipasquale and Oremland 1998; Pak and Bartha 1998).

We note that our modeling exercise assumes that K_m and K_{dm} were constant over the course of the summer, an assumption that we consider unlikely. It seems more likely that Hg methylation begins in sediments and evolves into the water column as summer progresses. Migration into the water column brings sulfate-reducing bacteria and other microbes closer to the source of important substrates (like sulfate) that have significant atmospheric sources. Proximity to epilimnetic sources of sulfate may confer an advantage to some sulfate-reducing bacteria that compete with methanogens for electron donors (Lovley and Klug 1986). In Devils Lake and Pallette Lake, anoxic conditions develop in June at the sediment–water interface, which is presumably where the zone of highest methylation potential would occur (C.S. Eckley and H. Hintelmann, unpublished data). In August, the zone of highest methylation potential (just below the oxycline) was detected at 14.0 m in Pallette Lake and 4.5 m in Devils Lake. Assuming that anoxia developed at a steady rate, the zone of highest methylation potential migrated roughly 1 m·month^{-1} up into the water column during the summer.

Our findings do not preclude the importance of methylation and demethylation in littoral sediments. Although the concentration of MeHg in surface waters is low and relatively constant over the summer, this apparent constancy may simply reflect much higher turnover driven by elevated

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Table 3. Rates of methylation, demethylation, and net MeHg accumulation in the hypolimnetic waters of Pallette Lake and Devils Lake during summer stratification.

Lake	Depth (m)	Measured K_m (day^{-1})	Fitted K_{dm} (day^{-1})	$T_{1/2}$ (days)	Initial concentration (ng·L^{-1})		Hg input (pg·L^{-1}·day^{-1})		Final concentration (ng·L^{-1})		MeHg:$Hg^{(II)}$	Concentration of sulfur species	
					MeHg	$Hg^{(II)}$	MeHg	$Hg^{(II)}$	MeHg	$Hg^{(II)}$		Sulfate (μmol·L^{-1})	Sulfide (μmol·L^{-1})
Pallette	14.0	0.043	0.101	7	0.08	0.67	0.2	2.2	0.22	0.75	0.29	22.1	0.9
Pallette	15.0	0.019	0.018	38	0.08	0.67	0.7	6.5	0.62	0.79	0.79	18.8	6.0
Pallette	16.0	0.021	0.015	46	0.08	0.67	0.7	6.7	0.72	0.71	1.02	16.5	13.1
Pallette	17.5	0.011	0.001	—	0.08	0.67	0.8	6.8	0.76	0.68	1.12	15.8	14.6
Devils	1.5	na	na	na	0.30	3.70	na	na	na	na	0.14	29.2	0.3
Devils	3.0	0.012	0.020	34	0.30	3.70	1.4	9.9	1.72	3.30	0.52	31.0	0.4
Devils	4.5	0.094	0.115	6	0.30	3.70	4.6	33.1	3.36	4.04	0.83	23.9	5.7
Devils	4.9	0.057	0.054	13	0.30	3.70	4.2	30.1	3.55	3.53	1.00	21.9	9.1
Devils	5.5	0.027	0.014	51	0.30	3.70	5.9	42.1	4.85	3.47	1.40	15.7	21.2
Devils	6.0	0.093	0.072	10	0.30	3.70	7.5	53.5	5.27	4.21	1.25	9.0	30.1

Note: The K_m was measured directly during 24-h incubations with ^{197}Hg. The K_{dm} was estimated indirectly using an iterative procedure to fit eqs. 3 and 4 to the observed ambient concentrations of MeHg at each depth stratum. Simulation started with observed concentrations of MeHg and $Hg^{(II)}$ in early spring and then MeHg and $Hg^{(II)}$ were incrementally added to the hypolimnion as summer progressed. Also shown is the observed ratio of MeHg:$Hg^{(II)}$ in the lake during late summer and changes in sulfur speciation with depth indicative of sulfate reduction in the water column. na, not available. $T_{1/2}$ is the half-life of MeHg, ln $2/K_{dm}$. MeHg and $Hg^{(II)}$ input is rate at which MeHg and $Hg^{(II)}$ enter the hypolimnion between spring and late summer, presumably via settling from the epilimnion.

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K_{dm} in surface waters owing to high light intensities and frequent mixing. However, even if MeHg is produced at high rates in epilimnetic sediments, the presence of an upper oxidized sediment layer may prevent MeHg from reaching the water (Gagnon et al. 1996; Regnell et al. 2001).

We conclude that Hg methylation and demethylation occur in the anoxic water columns of Pallette Lake and Devils Lake at rates sufficient to account for the observed accumulation of MeHg over summer. Both Devils Lake and Pallette Lake had peaks in methylation potential just below the oxycline, which concurs with previous findings for lakes in this region (Watras et al. 1995*a*; Mauro et al. 2002). In both lakes, the sulfide concentration increased below the oxycline, suggesting that sulfate-reducing bacteria are involved in the process of water column Hg methylation, and perhaps demethylation as well. Rates of methylation, demethylation, and MeHg accumulation were all substantially higher in the hypolimnion of the dark-water lake, perhaps because the higher concentrations of DOC and sulfate enhanced Hg methylating and demethylating microbial activity. In addition, lower light intensity might diminish rates of photodemethylation in the dark-water lake, explaining the higher buildup of MeHg in the dark-water lake.

Although wetlands are known to produce MeHg and export it to receiving waters via runoff, the results of this study suggest that the in situ production of MeHg can be a more important process even when wetlands play a dominant hydrologic role. Preliminary results from our ongoing mass balance studies of Devils Lake support this conclusion (C.J. Watras et al., unpublished data). Those data indicate that the stream emanating from the wetland was the major source of water, DOC, and sulfate to the lake during the year of this study. But even though concentrations of MeHg in the stream water were higher than those in the epilimnion of the lake (approaching 1 $ng \cdot L^{-1}$), and even though the wetland exported MeHg at a rate typical for northern wetlands (approximately 0.3 $\mu g \cdot m^{-2}$), the annual input of MeHg attributable to the wetland accounted for <20% of the MeHg accumulating in the entire water column of the lake. Most of the MeHg entering the epilimnion via stream inflow was lost via stream outflow during spring and summer. Thus, the dominant source of MeHg to both Devils Lake and Pallette Lake was in situ production. These findings agree with those of Sellers et al. (2001) who estimated the relative importance of all MeHg sources to be in-lake production >>> inflow from a brown-water lake with riparian wetlands >>> wet deposition > inflow from a clear-water lake or uplands.

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Effects of an increase in large wood on abundance and survival of juvenile salmonids (*Oncorhynchus* spp.) in an Oregon coastal stream

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Abstract: We examined the effect of an increase in large wood on the summer population size, smolt abundance, and freshwater survival of steelhead (*Oncorhynchus mykiss*), coastal cutthroat trout (*Oncorhynchus clarki clarki*), and coho salmon (*Oncorhynchus kisutch*). We examined these parameters for five brood years prior to the addition of wood and five brood years after in Tenmile Creek, a direct ocean tributary on the Oregon coast. Over the same time frame, a nearby reference stream, Cummins Creek, was also sampled for the same parameters. The input of large wood into Tenmile Creek resulted from a planned habitat restoration project in 1996 and an unplanned addition of wood from a winter storm the same year. Steelhead smolt abundance, steelhead freshwater survival, and coho salmon freshwater survival increased in Tenmile Creek after the input of large wood. Steelhead age-0+ summer populations and steelhead smolt populations increased in the reference stream, although steelhead freshwater survival did not. Coho salmon populations remained unchanged in the reference stream. Our results illustrate the potential shortcomings of the before-after-control-impact study design under field conditions and the potential for misinterpreting results had we employed a more modest sampling plan.

Résumé : Nous avons examiné les effets d'une augmentation des débris ligneux de grande taille sur la densité de la population en été, sur l'abondance des saumoneaux et sur la survie en eau douce chez la truite arc-en-ciel anadrome (*Oncorhynchus mykiss*), la truite fardée côtière (*Oncorhynchus clarki clarki*) et le saumon coho (*Oncorhynchus kisutch*). Nous avons suivi ces variables chez cinq cohortes annuelles de reproduction avant l'addition du bois et chez cinq cohortes après l'addition dans Tenmile Creek, un tributaire direct de l'océan sur la côte d'Oregon. Pendant la même période, nous avons mesuré les mêmes variables dans un cours d'eau témoin adjacent, Cummins Creek. L'addition de débris ligneux de grande taille résulte d'un projet planifié de restauration de l'habitat en 1996 et d'un apport imprévu de bois lors d'une tempête d'hiver la même année. L'abondance des saumoneaux de truites arc-en-ciel anadromes, la survie en eau douce des truites arc-en-ciel et la survie en eau douce des saumons coho ont augmenté à Tenmile Creek après l'addition de débris de bois de grande taille. Chez la truite arc-en-ciel anadrome du cours d'eau témoin, les populations d'été des poissons d'âge 0+ et les populations de saumoneaux ont augmenté, mais non la survie en eau douce. Les populations de saumons coho n'ont pas varié dans le cours d'eau témoin. Nos résultats illustrent les limites potentielles du plan d'expérience avant-après, témoin-impact dans les conditions de terrain et démontrent qu'il aurait été possible de mal interpréter nos résultats, si nous avions utilisé un plan d'expérience plus simple.

[Traduit par la Rédaction]

Introduction

In recent years, the role of large wood in streams of the Pacific Northwest has received considerable review (Harmon et al. 1986; Pearsons et al. 1992; Roni et al. 2002). Studies have documented the importance of large wood within the stream channel to slow bedload movement, deposit and sort gravels, scour pools, and increased nutrients through salmon carcass retention (Swanson et al. 1976; Cederholm and Peterson 1985; Ralph et al. 1994). Pools with large and complex accumulations of wood often show higher densities of rearing juvenile salmonids, particularly in winter, when storms routinely cause flooding in coastal streams (Bustard and Narver 1975; Nickelson et al. 1992*a*, 1992*b*). As our understanding of stream hydrology and habitat requirements of juvenile salmonids has improved, objectives and techniques for in-stream restoration have also evolved. However, few studies have quantified the changes in fish production resulting from freshwater restoration activities, and recent reviews have called for additional research (Smokorowski et al. 1998; Roni et al. 2002). Most studies have relied on estimates of fish numbers only near the restoration sites (Nickelson et al. 1992*b*; House 1996; Roni and Quinn 2001). While these types of studies are helpful in understanding the habitat requirements or preferences for the species in question, they were not designed to

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determine the total change in fish abundance in the entire stream.

Reeves et al. (1997) examined summer rearing populations and spring migrant populations of smolts of coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) from an entire stream before and after trees and boulders were added. Although steelhead smolt populations tended to be higher in post-treatment years, they observed no significant change in average population size. Solazzi et al. (2000) reported increased summer rearing populations, spring smolt populations, and overwinter survival of juvenile coho in two headwater streams of coastal Oregon after increasing the winter habitat compared with two nearby reference streams, where the habitat was not modified. They also observed increased spring migrants of age-1+ steelhead in the treatment streams after the habitat was modified.

In this paper, we examine the juvenile steelhead, coastal cutthroat trout (*Oncorhynchus clarki clarki*), and coho salmon populations from an Oregon coastal stream before and after a substantial input of large wood to the stream channel. Using an approach similar to that of Solazzi et al. (2000), we again examined the juvenile salmonid summer rearing populations and smolt populations for a treatment stream and a nearby reference stream; however, the treatment and reference streams examined in the present study are relatively small coastal watersheds and empty directly into the ocean. This allowed us to estimate summer rearing abundance and abundance of spring migrants for the entire watersheds. These streams support large populations of steelhead and coastal cutthroat trout, with coho salmon populations playing a smaller role. The instream restoration activities involved placing of whole trees in the active channel to create a series of large jams throughout the upper half of the treatment stream. The objective was to create numerous areas of large wood accumulations rather than creating specific pool types.

In February of 1996, an unplanned addition of large wood entered the main channel of the treatment stream during a large storm event. Natural debris jams were created from wood entering via debris slides from adjacent tributaries and from trees uprooted from the riparian area along the main channel. Because the flood impacts occurred in the same year as the planned in-stream restoration work (1996), the pretreatment and post-treatment design was unchanged. We redefined our evaluation to the broader question of how a sudden increase in large wood affects juvenile salmonid production and survival within a watershed. Thus, treatment effect refers to wood input into the watershed, some of which entered naturally through the February 1996 storm and some of which was placed into the stream channel as part of the restoration project in October 1996.

To determine changes in juvenile fish populations in the two streams, we estimated the summer rearing population for 10 years and the ocean migrant population for 12 years. Additional years of smolt trapping were required to determine the smolt production and freshwater survival of the age-0+ steelhead sampled in the ninth and tenth years of summer sampling. We hope that results from this type of long-term evaluation will provide fishery managers with a better understanding of the relationship between instream habitat and the freshwater production of juvenile salmonids.

Methods

Study area

Tenmile and Cummins creeks enter the Pacific Ocean on the central Oregon coast (Fig. 1). For our study, Tenmile Creek was the treatment stream and Cummins Creek was the reference stream. Because of the confining hillslope typography and the relatively high gradient of the watersheds, no estuary is present on either stream. The Tenmile Creek watershed encompasses approximately 60.7 km^2 and is located between the Cummins Creek and Rock Creek wilderness areas of the Siuslaw National Forest. The mainstem of Tenmile Creek is about 25.5 km in length. Two large tributaries, South Fork Tenmile Creek (3 km) and Wildcat Creek (2.1 km), also account for a significant amount of habitat within the watershed. The Tenmile Creek watershed consists almost entirely of the Yachats basalt formation (characteristic of the small coastal streams in the vicinity of Cape Perpetua), although Tyee sandstone (characteristic of most streams on the central Oregon coast) also exists at the extreme upper portion of the watershed (Baldwin 1992). The Cummins Creek watershed encompasses approximately 24.6 km^2 and enters the Pacific Ocean approximately 8 km north of the mouth of Tenmile Creek. Cummins Creek is approximately 11 km in length. The entire Cummins Creek drainage runs through the Yachats basalt formation.

The climate of the two watersheds is Pacific maritime characterized by wet winters and dry summers. Precipitation, primarily rain, ranges between 180 and 230 $cm \cdot year^{-1}$ (Loy 1976). Summer air temperatures range between 20 and 25 °C. Although winter air temperatures occasionally drop below freezing, most winter days range between 5 and 15 °C. Maximum summer water temperatures in both Tenmile and Cummins creeks reach 18 °C in late July and early August and drop to 4 °C at times during the winter.

The overstory riparian vegetation of both watersheds is a mixture of coniferous trees (Sitka spruce (*Picea sitchensis*), western redcedar (*Thuja plicata*), Douglas-fir (*Pseudotsuga menziesii*), and western hemlock (*Tsuga heterophylla*)) and hardwood trees (red alder (*Alnus rubra*) and bigleaf maple (*Acer macrophyllium*)). The understory consists primarily of salmonberry (*Rubus spectabilis*), salal (*Gaultheria shallon*), vine maple (*Acer circinatum*), and sword fern (*Polystichum munitum*).

Records indicate that Tenmile and Cummins creeks have consistently produced steelhead, cutthroat trout, and coho salmon. Chinook salmon (*Oncorhynchus tshawytscha*) spawn and rear in Tenmile Creek each year, although they are infrequently found in Cummins Creek. Chum salmon (*Oncorhynchus keta*) are found in Tenmile Creek in some years but were not observed in Cummins Creek during the study. We also observed Pacific lamprey (*Lampetra tridentata*), western brook lamprey (*Lampetra richardsoni*), eulachon (*Thaleichthys pacificus*), four species of sculpin (*Cottus asper, Cottus aleuticus, Cottus gulosus*, and *Cottus perplexus*), pacific giant salamanders (*Dicamptodon tenebrosus*), and tailed frogs (*Ascaphus truei*) in each basin.

To characterize fish rearing distribution and physical habitat within each watershed, we established four contiguous sampling reaches in the mainstem of Tenmile Creek and two sampling reaches in the largest tributaries, South Fork

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Fig. 1. Location of Tenmile Creek (treatment) and Cummins Creek (reference) on the central Oregon coast, United States. Numbers along channels refer to stream reaches. 1 mile = 1.609 km.

Tenmile and Wildcat creeks. In Cummins Creek, we established three contiguous sampling reaches (Fig. 1). Reach breaks corresponded to major changes in gradient and valley floor conformation. The lower reaches of each stream are broad valleys with constraining terraces, while upper stream reaches exhibit narrow valley characteristics. Channel morphology of all reaches is primarily constrained by alternating terraces and hillslopes. The surveys in each watershed represent the entire spawning and rearing distribution for salmon and the majority of habitat used by steelhead. Smaller tributaries account for a significant but undetermined amount of spawning and rearing habitat for cutthroat trout within each basin. Physical characteristics of the study reaches are provided in Table 1.

Description of habitat modification

Watershed restoration work in the Tenmile basin began in the summer of 1996 as a cooperative project with the US Forest Service (Siuslaw Forest) and local landowners. The US Forest Service decommissioned approximately 19 km of roads in the watershed, removing culverts and fill to decrease future landslides. Riparian areas were planted with approximately 2000 young conifer trees along approximately 1.6 km of stream. Some streamside riparian areas dominated by hardwood were thinned to increase the growth of existing conifers. In October of 1996, a Chinook helicopter using a hydraulic grappling hook attached to a 90-m cable transported 241 large conifer trees to the stream channel. About 200 of the trees (30–35 m length, 75 cm butt diameter) were felled on adjacent ridges and placed within the stream channel with limbs attached. The remaining trees were removed from two debris torrent deposits adjacent to Tenmile Creek. These trees often had rootwads attached but were generally shorter in length (15–20 m) than the felled trees. The trees were placed predominately in the upper half of the watershed. Eighty-eight trees were placed in the upper half of Reach 2, 133 trees were placed throughout Reach 3, and 20 trees were placed in the lower half of Reach 4. Most placements consisted of three to eight large trees to produce accumulations of large wood and were located near the upper or lower entrances of old side channels or in natural bends where large debris would logically accumulate. Trees were not cabled or attached, although they were sometimes wedged between existing live trees in the riparian area to increase stability. Two numbered aluminum tags were attached to each habitat tree to allow us to identify trees in post-restoration surveys.

Several months prior to the instream tree placement work in the upper watershed, a large storm caused additional large wood to enter the main channel of Tenmile Creek. The amount of large wood deposited by the storm was similar to the amount placed by the restoration work; however, wood deposited by flooding was confined primarily to the lower half of the watershed, while the trees flown in as part of the restoration work were concentrated in the upper half of the basin. This same storm also caused new wood to enter the Cummins Creek watershed. Much of this wood debris was deposited in the upper portion of the basin above most

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Table 1. Physical characteristics of Tenmile and Cummins creeks.

Stream	Stream length (km)	Mean summer wetted width (m)	Mean active channel width (m)	Average gradient (%)
Tenmile (60.7 m^2)[a]				
Mainstem Reach 1	3.7	9.6	23.1	1.0
Mainstem Reach 2	8.8	9.2	26.3	1.1
Mainstem Reach 3	3.7	6.8	17.4	1.9
Mainstem Reach 4	3.2	4.2	10.3	4.1
South Fork	3.0	4.1	8.5	5.3
Wildcat	2.1	4.1	9.0	4.5
Cummins (24.6 m^2)[a]				
Mainstem Reach 1	4.3	6.1	11.5	1.7
Mainstem Reach 2	4.6	5.1	9.0	2.5
Mainstem Reach 3	1.8	3.4	4.3	5.1

[a]Basin area.

salmon and steelhead spawning and rearing areas (on average only 5% of the steelhead and <1% of the coho summer populations were found in this area).

Habitat surveys

During August and September, we used the Hankin and Reeves (1988) methodology to estimate the amount of available summer habitat. We conducted summer habitat surveys in Tenmile Creek in 1991, 1993, and 1995–2000. In Cummins Creek, we conducted habitat surveys in 1991, 1992, 1994, and 1995–2000. We also conducted winter habitat surveys in Tenmile Creek in 1993 and 1997 and in Cummins Creek in 1991 and 1997. Habitat was classified using the methods of Bisson et al. (1982) as modified by Nickelson et al. (1992*a*). Surface area for each habitat unit was visually estimated, and every tenth unit was measured to calibrate the visual estimates. We classified the substrate in each habitat unit (Moore et al. 1997) and measured the maximum depth of each pool. We counted the number of pieces of wood (≥ 3 m in length and ≥ 15 cm in diameter) in the active channel once during the pretreatment period (1993) and once during the post-treatment period (1999). Key pieces of wood were defined as pieces ≥ 12 m in length and ≥ 60 cm in diameter (Moore et al. 1997).

Estimating summer fish populations

Estimates of the number of age-0+ steelhead and age-0+ coho salmon rearing in each stream reach were made each year during August and early September. Stream reach population estimates were combined to obtain an abundance estimate for the entire stream.

To estimate the number of fish rearing in pool habitat within each reach, divers counted the number of each species in every third pool. We electrofished in at least five pools in each stream reach to calibrate and adjust the diver counts. The mean of the adjusted values was then multiplied by the total number of pools in the reach (Hankin and Reeves 1988). Because snorkel estimates are impractical in shallow habitat, we estimated the mean density of each species for a subset of glide, riffle, and rapid habitats by electrofishing. For each habitat type, we then multiplied this mean density by the surface area of this habitat type in the entire stream reach (Hankin 1984). We generally sampled at least 10 of each shallow-water habitat (riffles, rapids, and glides) in each stream reach to estimate densities in these habitat types.

For the electrofishing sampling, we estimated the number of each species in each sample unit using either a mark–recapture estimate (Chapman 1951) or a removal estimate with two or more passes (Seber and LeCren 1967). Mark–recapture estimates were generally used in pool habitat with high wood complexity or with special sampling problems where removal estimation methods have been shown to be less accurate (Rodgers et al. 1992). Every habitat unit was blocked by seines on both ends and sampled for juvenile salmonids using 1000-V direct current backpack electrofishers. Criteria for sampling intensity were established to control the size of the confidence interval derived from the population estimate and to prevent exposing the fish to unnecessary repeated electrofishing.

Estimating the number of downstream migrants

We estimated the number of downstream-migrating juvenile steelhead, cutthroat trout, and coho salmon in each stream each spring for 12 years using rotating screw traps. In each stream, traps were located near the stream mouth, just before the stream emptied into the Pacific Ocean. Traps were operated from the first week in March until catches diminished to low levels in mid- to late June. Traps operated continuously and were normally checked and cleared of fish and debris once a day, although traps often required constant attention during storm events. Fish were anesthetized with tricaine methanesulfonate (MS 222) and enumerated by species and size class. A sample of 25 lengths was taken on each size group of each species each week. Coho salmon were identified as fry or smolts (≥ 80 mm). Trout fry (< 60 mm) were not differentiated to the species level. Additional size classes were 60–89, 90–119, and ≥ 120 mm for steelhead and 60–89, 90–119, 120–159, and ≥ 160 mm for cutthroat trout. Only fish in the largest size class for each species showed signs of smoltification (silvering, loss of condition). Therefore, we defined these fish as smolts in our analyses.

To estimate trap efficiency, we daily marked up to 25 fish of each species and size group with either a small clip on

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their caudal fin (fish < 90 mm) or an injection of dye at the base of the ventral fin using a Panjet injector (Wright Health Group Ltd., Dundee, Scotland) (fish ≥ 90 mm). Marked fish were released at dusk approximately 200 m upstream of the traps. For each species and size group, weekly trap efficiency estimates were calculated by dividing the number of marked fish recaptured by the number of marked fish released. The total number of unmarked fish captured during the week was then divided by the weekly trap efficiency to estimate the total number of fish of each species migrating past the trap during the week. Weekly estimates were summed to estimate the total number of migrants of each species passing the trap each spring.

Estimating age composition of juvenile salmonids

Each year, scale samples were taken on juvenile steelhead during the summer rearing period and spring migration period to determine age composition. In the summer (August), scale samples were taken from 20 fish per 1-cm size interval. During the spring migration period (March–June), scale samples were taken from 10 fish per 1-cm size interval each month. For each sampling period, the age composition of each 1-cm size interval was applied to the length frequency of the population to determine the age composition of the entire population. This information was then used to partition each calendar year's population estimate into the appropriate brood years for subsequent analysis. For example, each calendar year, the steelhead smolt population was composed of smolts from three different brood years (age-1+, age-2+, and age-3+ smolts). To determine the number of steelhead smolts from a particular brood year (1992, for example), we added the age-1+ smolts from the spring of 1993, the age-2+ smolts from the spring of 1994, and the age-3+ smolts from 1995.

Study design and analysis

We designed the study using a before-after-control-impact (BACI) design suggested by Stewart-Oaten et al. (1986) to assess changes in habitat and fish parameters, a design that we had employed in an earlier study (Solazzi et al. 2000). However, after examining the data, it became evident that the pairing of the treatment and reference streams was not appropriate for this data set. This approach assumes that the reference stream will "track" the treatment stream. A change in this tracking relationship after the treatment impact provides evidence for an effect. In this study, the reference stream did not track well with the treatment stream. The rearing density and overall population size of age-0+ steelhead populations in the treatment stream remained at a high and constant level throughout the study. Alternatively, the populations of age-0+ steelhead in the reference stream were low relative to the available habitat in the first years of the study but increased steadily over time, presumably from increased numbers of spawning adult steelhead. Such differences in trends over time between treatment and reference violate the assumptions inherent in the BACI analysis and therefore may result in misleading significance (Stewart-Oaten et al. 1986; Smith et al. 1993). Additionally, in one year of the study, no age-0+ coho salmon were observed in the reference stream, which creates an additional problem if the ratio of treatment to control is used for analysis (Smith et al. 1993).

Because of these difficulties, we analyzed the data separately for each stream. For each habitat and fish population parameter, we used a *t* test to compare the means for the pretreatment and post-treatment periods. For each fish parameter, our null hypothesis was that the post-treatment mean was not different from the pretreatment mean; thus, we used a two-tailed test for each comparison. We excluded broods from each analysis that would have spent part of their lives in pretreatment years and part in post-treatment years (transition years). For consistency, brood year was designated as the year of age-0+ rearing for both steelhead and coho salmon. For habitat parameters, our null hypothesis was the post-treatment period was not greater than the pretreatment period because we expected the addition of large wood to create more pool habitat. Thus, we used a one-tailed test for these comparisons. The layout for the analysis for each species and life history stage and habitat attribute is provided in Table 2.

For steelhead and coho salmon, we determined whether age-0+ summer abundance, spring smolt abundance, or freshwater survival changed between the pretreatment and post-treatment periods for the 1991–2000 broods. Because we did not have accurate estimates of the number of adult spawners for each brood year, we defined freshwater survival as the percentage of age-0+ fish that survived to smolt. We did not analyze freshwater survival for coho salmon in Cummins Creek because in four of the 10 brood years, the summer population was so low that reliable estimates of survival could not be made. This also occurred in the 1998 survival estimate of coho salmon in Tenmile Creek, and we removed that brood from the analysis of freshwater survival.

For cutthroat trout, we determined whether the spring sea-run smolt abundance changed between the pretreatment and post-treatment periods. We analyzed the cutthroat trout smolt data by calendar year, rather than by brood year, because we found it difficult to age many of the upper year-class cutthroat by scale analysis. Unlike steelhead and salmon in these streams, not all of the cutthroat trout migrate to the ocean. Estimates of sea-run cutthroat smolts are therefore not a complete estimate of year-class strength. Scale analysis did suggest that most sea-run cutthroat smolts migrated to the ocean after 3 years of freshwater residence, although some age-2 and age-4 migrants were also observed.

Results

Habitat

In Tenmile Creek (treatment stream), the number of key pieces of wood (wood pieces ≥12 m in length and ≥ 60 cm diameter) increased from 7.1 pieces·km^{-1} in the pretreatment count done in 1993 to 38.8 pieces·km^{-1} in the post-treatment count completed in 1999. The increase was observed in all four reaches in the mainstem of Tenmile Creek, where the number of key pieces increased 2.5 times in Reach 1, 5.7 times in Reach 2, 7.4 times in Reach 3, and 10.4 times in Reach 4 (Fig. 2). Placement of large trees during habitat restoration work accounted for all of the increase in large wood in Reach 3, while the storm-induced input of wood accounted for the additional large wood in Reach 1. The in-

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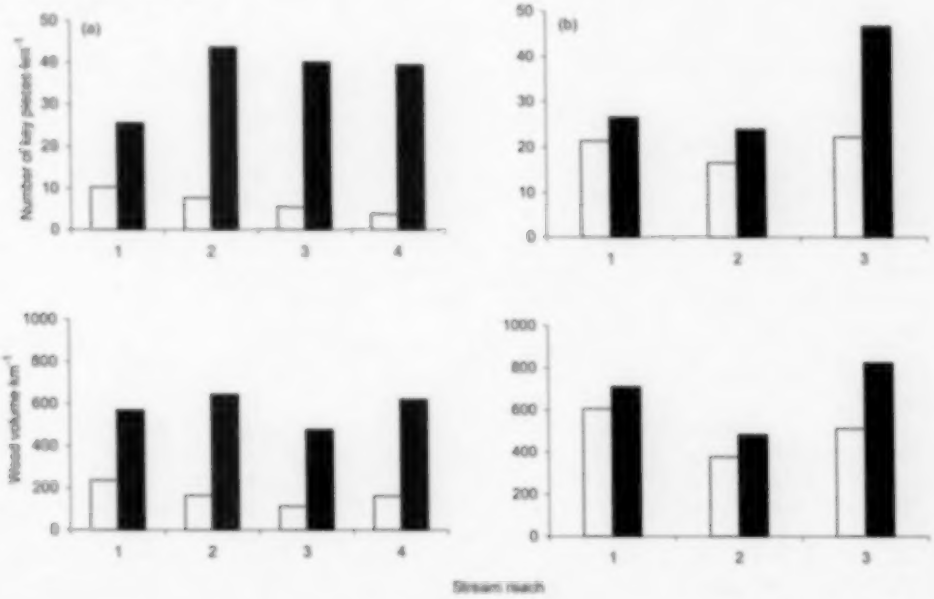
Table 2. Analytical layout of the habitat and population parameters.

Parameter	Data collection period		
	Pretreatment years	Transition years	Post-treatment years
Physical habitat			
Summer			
Tenmile Creek	1991, 1993, 1995	1996	1997–2000
Cummins Creek	1991, 1992, 1994–1995	1996	1997–2000
Winter			
Tenmile Creek	1993		1997
Cummins Creek	1991		1997
Steelhead age-0+ summer population	1991–1995	1996	1997–2000
Steelhead smolt population	1991–1994	1995–1996	1997–2000
Steelhead freshwater survival	1991–1994	1995–1996	1997–2000
Coho salmon summer population	1991–1995	1996	1997–2000
Coho salmon smolt population	1991–1995	1996	1997–2000
Coho salmon freshwater survival	1991–1995	1996	1997, 1999–2000
Cutthroat smolt population	1992–1995	1996–1997	1998–2001

Note: Transition years for fish populations refer to years that were not used in the analyses because juveniles spent a portion of their freshwater rearing in both pretreatment and post-treatment years. For habitat comparisons and cutthroat trout (*Oncorhynchus clarki clarki*) population comparisons, years refer to calendar year. For steelhead (*Oncorhynchus mykiss*) and coho (*Oncorhynchus kisutch*) salmon population comparisons, years refer to brood years. For consistency, brood year is based on the year of age-0+ summer rearing for both steelhead and coho salmon. Total count of wood pieces was completed once in the pretreatment years (1993) and once in the post-treatment years (1999).

Fig. 2. Comparison of the number of key pieces of large wood (pieces 12 m in length and 60 cm in diameter) and the total volume (m^3) of wood in (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference) before (1993) and after (1999) wood input in 1996. Increases in wood in Tenmile Creek resulted from a February 1996 storm event and an October 1996 instream habitat restoration project. Increases in wood in Cummins Creek resulted only from the February 1996 storm. Open bars represent pretreatment years and solid bars represent post-treatment years.



crease in Reach 2 and Reach 4 resulted from both the restoration trees and the flood impacts. In Cummins Creek (reference stream), key pieces of wood increased from 19.5 pieces·km^{-1} before the February 1996 storm event (1993 survey) to 28.9 pieces·km^{-1} after the storm (1999 survey). However, most of this increase was in the upper watershed (Reach 3), above most of the spawning and rearing of anadromous fish (Fig. 2). Estimates of total volume of wood (≥ 3 m in length and ≥ 15 cm in diameter) also increased throughout the mainstem of Tenmile Creek during the post-treatment period (167–593 m^3). Estimates of total volume of wood in Cummins Creek showed a smaller increase (494–

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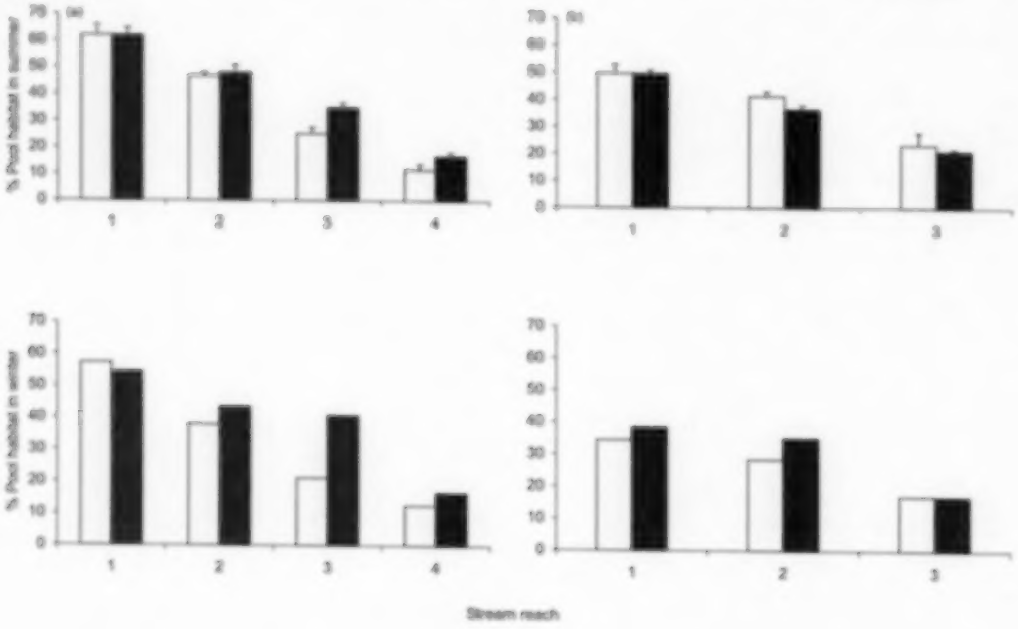
Fig. 3. Comparison of the mean percentage of summer and winter pool surface area (pool surface area/total wetted surface area) in (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference) before and after wood input in 1996. Increases in wood in Tenmile Creek resulted from a February 1996 storm event and an October 1996 instream habitat restoration project. Increases in wood in Cummins Creek resulted only from the February 1996 storm. Open bars represent pretreatment years and solid bars represent post-treatment years. For summer habitat, error bars indicate standard error. Winter habitat was surveyed only once in each period; thus, no error bars are provided.



633 m^3), observed primarily in the upper portion of the watershed (Fig. 2).

In the upper portion of Tenmile Creek, the percentage of summer wetted surface area classified as pool habitat increased during the post-treatment phase of the study (Reach 3, one-tailed t test: $p = 0.010$; Reach 4, $p = 0.040$), while Reach 1 and Reach 2 showed no change ($p = 0.48$ and $p = 0.37$, respectively) (Fig. 3). More of the summer surface area of pool habitat in Tenmile Creek was associated with deep pools (maximum depth ≥ 1 m) in the post-treatment period. In Reach 3, where most of the habitat restoration trees were placed in the stream channel, the percentage of pool habitat associated with pools ≥ 1 m deep increased from an average of 21% in the pretreatment years to an average of 36% in the post-treatment years (one-tailed t test: $p = 0.046$). The reference stream, Cummins Creek, did not show a change in the percentage of summer stream surface area classified as pool habitat between pretreatment and post-treatment years.

Winter habitat surveys were completed once in the pretreatment period and once in the post-treatment period during periods of comparable flows. These surveys indicate that total surface area of mainstem winter habitat increased in Reach 3 of Tenmile Creek following treatment (Fig. 4), and much of this increase was associated with pool habitat (Fig. 3). In Cummins Creek, winter habitat surveys did not show an increase in the surface area of total habitat or pool habitat. We also observed an increase in side-channel habitat in Reach 2 and Reach 3 in Tenmile Creek after treatment, while side-channel habitat in Cummins Creek remained relatively constant (Fig. 4).

Changes in substrate composition were not detectable at the reach level for either stream during the course of the study. Major changes in substrate composition were observed, however, at individual wood jams (both natural and restoration sites) as gravels were deposited above and below the jams during high-water events.

Fish populations

Steelhead

The summer population size of age-0+ steelhead in the treatment stream (Tenmile Creek) did not change significantly between pretreatment and post-treatment years ($p = 0.946$) (Fig. 5). In the reference stream (Cummins Creek), there was a significant increase in the age-0+ steelhead summer rearing populations from the pretreatment to the post-treatment period ($p = 0.001$) (Fig. 5). Estimates of steelhead smolt abundance in the spring increased significantly in both Tenmile Creek ($p = 0.016$) and Cummins Creek ($p = 0.035$) between the pretreatment and post-treatment years (Fig. 5). Freshwater survival increased significantly in Tenmile Creek from the pretreatment to the post-treatment years ($p = 0.011$) but did not change significantly in Cummins Creek ($p = 0.18$) (Fig. 5).

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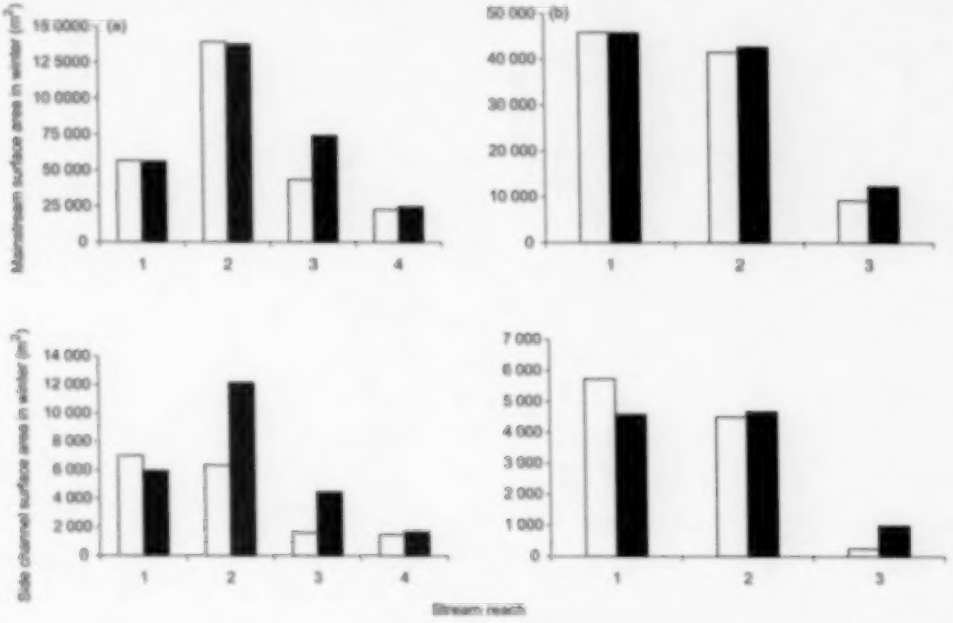
Fig. 4. Comparison of winter wetted surface area of mainstem and side-channel habitat in (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference) before and after wood input in 1996. Pretreatment winter habitat surveys (open bars) were completed in the winter of 1991–1992 in Cummins Creek and 1993–1994 in Tenmile Creek. Post-treatment winter habitat surveys (solid bars) were completed in both streams in the winter of 1997–1998.



Cutthroat trout

The number of searun cutthroat smolts migrating to the ocean in the spring increased significantly in both Tenmile Creek ($p = 0.0015$) and Cummins Creek ($p = 0.0075$) (Fig. 6) between pretreatment and post-treatment periods. No estimates of age-0+ population or of freshwater survival were made for cutthroat trout.

Coho salmon

There was no significant change in the summer rearing populations of age-0+ coho salmon in Tenmile Creek ($p = 0.080$) or Cummins Creek ($p = 0.324$) between pretreatment and post-treatment years. There was also no significant change in the number of coho salmon smolts leaving Tenmile Creek ($p = 0.496$) or Cummins Creek ($p = 0.791$) between pretreatment and post-treatment years (Fig. 7). However, juvenile coho salmon did show a significant ($p = 0.005$) increase in freshwater survival in Tenmile Creek (Fig. 7). Coho salmon freshwater survival was not calculated for Cummins Creek because low summer population sizes in several years made it difficult to accurately determine the percentage that survived.

Discussion

The addition of wood to Tenmile Creek by the February 1996 storm event and the October 1996 habitat restoration activities increased the number of large key pieces of wood to levels observed in the reference stream, Cummins Creek. This resulted in an increase in the percentage of surface area of pool habitat and pool depth in the upper two reaches of Tenmile Creek. Winter side-channel habitat also increased in Reach 2 and Reach 3 of Tenmile Creek after the input of large wood. A similar response to the physical habitat of other Pacific Northwest streams has been observed after the addition of large wood (Crispin et al. 1993; Reeves et al. 1997; Solazzi et al. 2000).

Our results suggest that these habitat changes resulted in significantly higher survival for juvenile steelhead and coho salmon in Tenmile Creek in the post-treatment period. The increased survival resulted in steelhead smolt abundance that averaged 2.5 times higher in the post-treatment period than in the pretreatment period. In the reference stream, Cummins Creek, the post-treatment steelhead smolt abundance averaged 1.6 times higher than in pretreatment years but resulted from an increasing population size of age-0+ steelhead in the stream over the 10-year period rather than an increase in freshwater survival, as was observed in Tenmile Creek.

Results from other studies examining the effect of increased instream structure on juvenile steelhead have been inconclusive. The addition of boulders and placement of rock-filled gabions in an Oregon coastal stream led to an increase in the summer rearing population of juvenile steelhead (House and Boehne 1985), but no increase was observed in age-0+ or age-1+ steelhead populations after the addition of large wood to a Washington stream (Cederholm et al. 1997). In Washington and Oregon streams, steelhead and cutthroat populations did not change near large woody debris placement projects compared with control reaches during the summer but increased during the winter (Roni and Quinn 2001). However, these studies measured steelhead population size at the reach level and did not attempt to estimate

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Fig. 5. Age-0+ steelhead (*Oncorhynchus mykiss*) summer rearing populations, spring smolt population, and freshwater survival for the 1991–2000 brood years in (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference). Freshwater survival is defined as the percentage of age-0+ steelhead that survive to smolt. Open bars represent pretreatment brood years, hatched bars represent transition brood years, and solid bars represent post-treatment brood years.

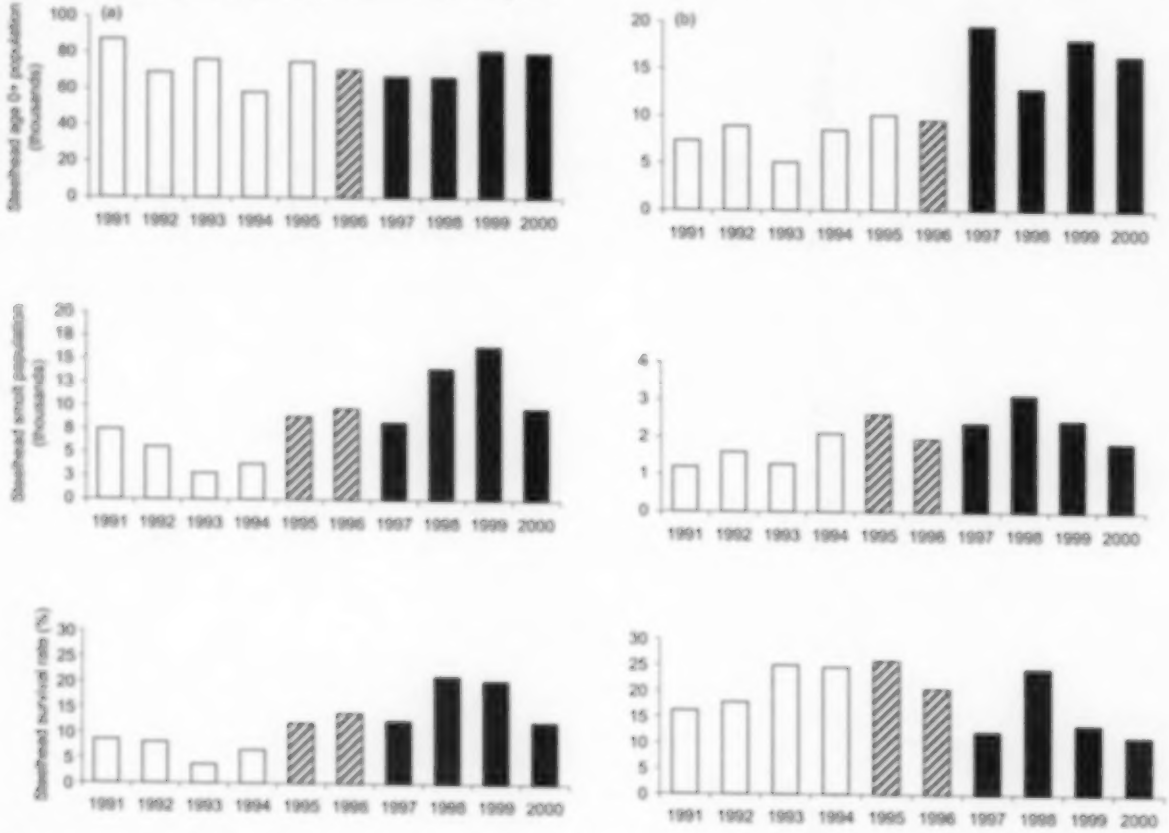
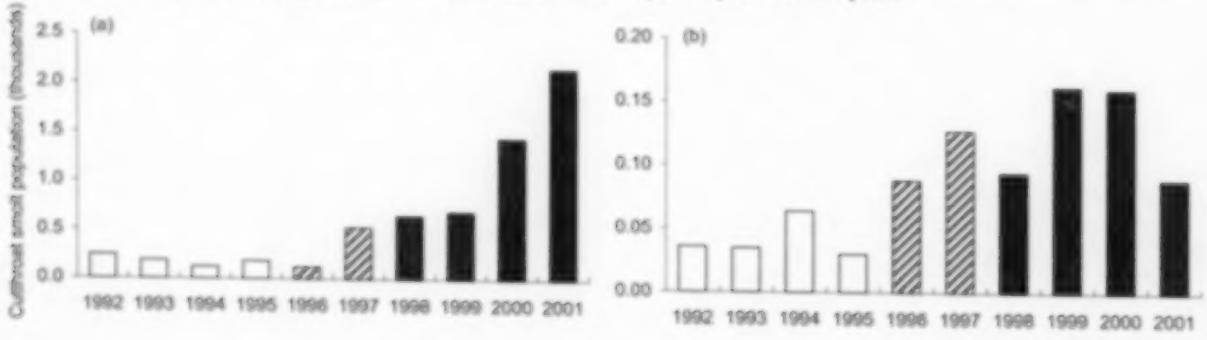

(a)
(b)
Steelhead age 0+ population (thousands)
Steelhead smolt population (thousands)
Steelhead survival rate (%)
1991 1992 1993 1994 1995 1996 1997 1998 1999 2000

Fig. 6. Sea-run cutthroat trout (*Oncorhynchus clarki clarki*) spring migrant populations for (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference) for calendar years 1992–2001. Cutthroat smolts are defined as fish 160 mm. Open bars represent pretreatment years, hatched bars represent transition years, and solid bars represent post-treatment years.



smolt abundance for the entire stream. Solazzi et al. (2000) did not observe an increase in the abundance of juvenile steelhead in the summer in two Oregon coastal streams after habitat restoration activities but did observe an increase in the number of spring migrant steelhead (> 90 mm). However, most of these migrants were not smolts on their ocean migration but age-1+ fish moving downstream for another year of rearing. Reeves et al. (1997) observed a 28% increase in the mean number of steelhead smolts leaving Fish Creek (Clackamas River, Oregon) after habitat restoration, al-

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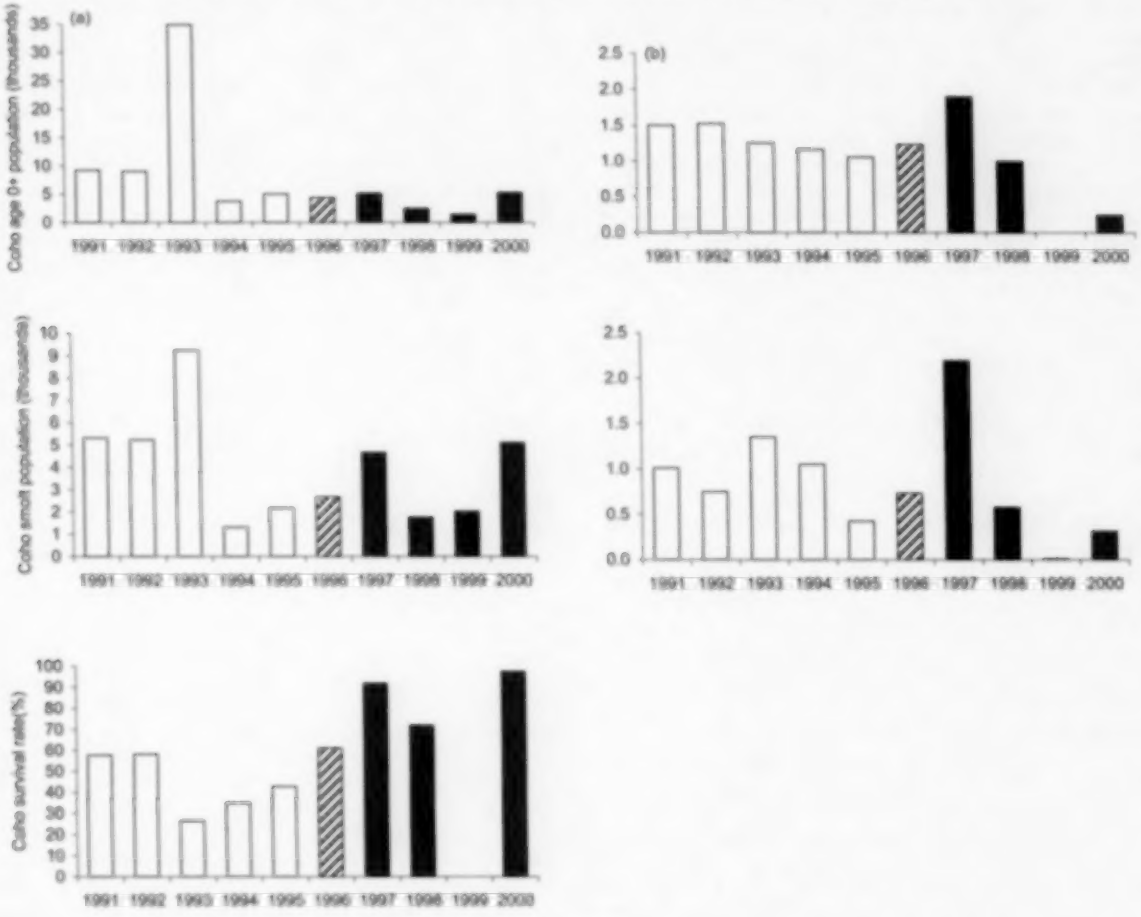
Fig. 7. Age-0+ coho salmon (*Oncorhynchus kisutch*) summer rearing populations and spring migrant populations for the 1991–2000 brood years in (*a*) Tenmile Creek (treatment) and (*b*) Cummins Creek (reference). Freshwater survival is also shown for Tenmile Creek. Freshwater survival is defined as the percentage of age-0+ coho salmon that survive to smolt. Open bars represent pretreatment brood years, hatched bars represent transition brood years, and solid bars represent post-treatment brood years.


(a)
Coho age 0+ population (thousands)
(b)
Coho smolt population (thousands)
Coho survival rate(%)
1991 1992 1993 1994 1995 1996 1997 1998 1999 2000

though the increase was statistically insignificant because of the variation in the annual number of migrants both before and after treatment.

Abundance of steelhead smolts is inherently difficult to measure in many coastal streams because many juvenile steelhead leave their natal streams after 1 year of rearing and spend another year of rearing in larger streams. Therefore, if study streams are selected in smaller tributaries in the upper portions of a watershed, a complete estimate of steelhead smolt production is not possible. Because Tenmile and Cummins creeks are both direct ocean tributaries, we avoided this problem by placing our smolt traps near the stream mouths, where the streams entered the Pacific Ocean.

Smolt trapping in the spring showed significant increases in sea-run cutthroat migrants in both Tenmile and Cummins creeks in the post-treatment period of the study. Solazzi et al. (2000) reported finding no detectable differences in summer cutthroat numbers but observed increases in the number of migrants after restoration work on one of two Oregon coastal streams. Because we do not know the age-0+ population size of cutthroat trout in Tenmile and Cummins creeks, it is difficult to interpret the results of increased cutthroat smolt production during the post-treatment period. While habitat changes may have influenced cutthroat smolts in the post-treatment period in Tenmile Creek, more restrictive trout fishing regulations were placed on all Oregon coastal streams in the mid-1990s and could also be partially responsible for the increased number of sea-run cutthroat migrants that we observed.

No significant change in the coho salmon summer rearing population size or spring smolt abundance was observed in either stream during the study. Summer rearing densities of juvenile coho salmon in both Tenmile and Cummins creeks were low, generally <0.1 fish·m^2 pool surface area^{-1}. Rearing densities of age-0+ coho salmon in Oregon coastal streams with adequate adult spawning the previous winter usually average close to 1.5 fish·m^2 pool surface area^{-1} (Nickelson et al. 1992*a*). The low rearing densities measured in this study

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were likely the result of low adult spawner escapement to these and other Oregon coastal streams during the 1990s.

These results illustrate the problem of trying to evaluate effects of habitat restoration on anadromous fish populations in streams when population sizes are low. While the habitat changes may result in an increase in the potential rearing capacity, short-term studies (and this study was relatively long term) may not observe increases if fry abundance is insufficient to exploit all available habitat. In addition, when streams have low numbers of adult spawners, small changes in adult spawners can result in a large variation in juvenile abundance, regardless of habitat restoration activities. If population levels are low and freshwater survival is high because of adequate habitat and little density-dependent mortality, field sampling techniques may not be sensitive enough to detect changes in freshwater survival. This was particularly true in this study for coho salmon in Cummins Creek, where the confidence intervals associated with summer population estimates and spring smolt estimates of coho salmon often overlapped.

Although we observed a significant increase in the freshwater survival of juvenile coho salmon in Tenmile Creek, summer rearing densities were generally lower in the post-treatment period than in the pretreatment period, and less density-dependent mortality may have contributed to the observed increase in freshwater survival in the post-treatment period. In particular, the summer rearing population of age-0+ coho salmon for the 1993 brood in Tenmile Creek was much higher than in other years and was undoubtedly subjected to more density-dependent mortality during the winter. This may have biased the comparison of survival between pretreatment and post-treatment years.

Solazzi et al. (2000) observed increased freshwater survival of coho juveniles in East Creek (Nestucca River, Oregon) and Lobster Creek (Alsea River, Oregon) after instream restoration projects. Freshwater survival was higher in this study than observed in East and Lobster creeks, presumably because of the low rearing densities observed in Tenmile Creek. Others have also documented positive responses to juvenile coho populations after instream structure was added to Pacific Northwest streams (Nickelson et al. 1992*b*; Cederholm et al. 1997; Roni and Quinn 2001). However, Reeves et al. (1997) did not observe a change in summer rearing populations or smolt populations of coho salmon after restoration activities in Fish Creek (Clackamas River, Oregon).

This study was designed in an approach outlined by Stewart-Oaten et al. (1986), where we sampled multiple years before and after treatment (wood input) and also used an adjacent stream as a reference to isolate the impacts of the habitat modification on fish populations. Solazzi et al. (2000), using this study design, documented how results could be misinterpreted without the inclusion of a reference stream. In this study, using Cummins Creek as a reference stream had unexpected consequences that made interpretation of steelhead numbers using a standard BACI design inappropriate. Cummins Creek initially seemed like a good reference stream. Although smaller in size than Tenmile Creek, it was in close proximity, similar in geology, and had a similar history of fish management to the treatment stream. In addition, because the Cummins Creek watershed is designated as a wilderness area, we were assured that land use activities within the watershed would remain unchanged during the course of the study. Still, Cummins Creek eventually proved to be an inappropriate reference stream because the trend of age-0+ steelhead populations was different from what we observed in the treatment stream.

Ideally, both the treatment and reference streams would have an adequate number of adult spawners to seed the available rearing habitat with emerging fry each spring. This would ensure that the summer rearing populations and smolt populations reflect the production potential of the available habitat. It would also minimize the variation in density-dependent mortality between years and between treatment and reference streams. Large changes in density-dependent mortality can minimize our ability to determine if habitat changes result in changes in freshwater survival. If sufficient numbers of age-0+ juveniles do not occur to seed the available rearing habitat, the treatment stream and reference stream should at least show the same trend in the pretreatment period (Smith et al. 1993). In this study, however, the rearing densities of age-0+ steelhead were high and constant in the treatment stream (Tenmile Creek), while the rearing densities in the reference stream (Cummins Creek) were initially low and increased throughout the 10-year study. In Tenmile Creek, the rearing densities of age-0+ steelhead averaged 0.3 fish·m^{-2} in riffles and 0.4 fish·m^{-2} in pools. These densities are similar to those observed in other Oregon coastal streams with established steelhead populations (S.L. Johnson, unpublished data). At the beginning of the study, the rearing densities in the reference stream were substantially lower (0.1 fish·m^{-2} in riffles and 0.2 fish·m^{-2} in pools) but increased over the course of the study, presumably from an increase in adult spawners. Because of this difference in the initial seeding levels of fry between the two streams, the increases in smolt production exhibited by both streams occurred for different reasons. The steelhead smolt abundance in the reference stream increased because initial seeding of fry increased, resulting in more juveniles rearing in the stream and eventually more smolts migrating to the ocean. Survival of age-0+ steelhead to smolt (primarily age 2+), however, remained unchanged for the reference stream over the course of the study. In the treatment stream (Tenmile Creek), the age-0+ steelhead population size remained constant over time, but the smolt abundance increased because freshwater survival increased.

Our results illustrate the difficulty in obtaining definitive conclusions when trying to determine the effect of habitat changes on juvenile salmonid abundance. Various sampling designs have been proposed to assess ecological impacts. (Hicks et al. 1991; Osenberg et al. 1996; Roni et al. 2003). The BACI-type design seems to present a logical approach to evaluate the effect of habitat restoration projects on salmon populations and has been used successfully in the past (Solazzi et al. 2000). This approach allows for a direct comparison of fish abundance and survival in an entire stream before and after a treatment or impact. A successful evaluation, however, is difficult to achieve. Interannual variation in juvenile salmonid populations is often large because of changes in adult spawner abundance or environmental condi-

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tions. To understand this variation requires a study design with pretreatment and post-treatment periods of at least 4 or 5 years for each period to attain even a minimal sample size (Roni et al. 2003). In our study, we removed the middle two brood years from our comparisons of steelhead smolt abundance because these fish spent part of their freshwater residence in the pretreatment period and part in the post-treatment period. Thus, although we sampled steelhead populations for 10 brood years, we could only use eight years to compare pretreatment and post-treatment smolt abundance and survival. Maintaining continuity in funding, personnel, and methods is always a challenge with long-term studies. Unforeseen changes in land use activities, changes in fishing regulations, or large impacts from floods or droughts that affect treatment and reference streams disproportionately also complicate evaluation. Resource limitations have rarely allowed researchers to include true replication by sampling multiple treatment and reference watersheds simultaneously. Underwood (1996) argued convincingly that including multiple reference sites with a given treatment site would alleviate many of the problems associated with comparing a single treatment and reference as outlined by Stewart-Oaten et al. (1986). In future studies, this design would be an improvement if additional appropriate reference sites could be identified and adequate funding could be secured.

When many species are sampled as part of a BACI design, Smith et al. (1993) suggested dropping species from the analysis that do not show consistent trends between treatment and reference sites and concentrate the analysis on species that do show consistent trends. This solution is not suitable when sampling fish populations in the Pacific Northwest, where species diversity is naturally low and salmonid species are often the primary concern. In our situation, we felt that it was most appropriate to acknowledge that other factors complicated the direct comparison of treatment and reference streams and analyze the data separately for each stream.

Murtaugh (2000) pointed out that large-scale ecological studies often have little or no replication, yet many of these studies have contributed to our understanding of ecological processes. In a recent review of the BACI design, Conquest (2000) concluded that sometimes the best interpretation could be accomplished by staring hard at good graphs and using process knowledge in one's arguments. In this study, even with the complications of incorporating these data into a typical BACI design, we did increase our understanding of how these fish populations reacted to the change in the amount of large wood input into the Tenmile Creek watershed. Our understanding of how steelhead freshwater survival changed in Tenmile Creek would not have been possible without the intensive sampling required to measure both the summer population size of age-0+ fish and their subsequent smolt abundance for each brood year. Had we chosen a less intensive approach, perhaps sampling at only one life history stage, resources could have been reallocated to include additional streams in the study design. However, we would not have gained the same insights into the changes in total population size and freshwater survival. For example, if we had only sampled the steelhead smolt populations migrating from the two streams each spring, we would have concluded that the Tenmile Creek steelhead population was unchanged relative to Cummins Creek (steelhead smolt production increased in both streams). Only by sampling both the summer rearing populations and the spring smolt populations were we able to understand what happened in these two streams over the course of this study.

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Modeling feeding processes: a test of a new model for sea bream (*Sparus aurata*) larvae

Konstadia Lika and Nikos Papandroulakis

Abstract: An organism's feeding rate is governed by constraints imposed by processes associated with consumption. We present a general feeding model that incorporates encounter, successful pursuit, handling, and digestion in one functional representation where we treat digestion as a parallel process. The model produces type II functional response curves. However, the asymptotic maximum feeding rate is determined by the sum of the time spent for handling and digesting a prey minus the gain in time, since the digestion process is parallel to the handling process. We use our model in combination with existing models of encounter, successful pursuit, and digestion to evaluate the feeding rate of fish larvae. We test the model against experimental data for sea bream (*Sparus aurata*) larvae and find a very close quantitative correspondence between predictions and experiments. Sensitivity analysis shows that for the early developmental stages, the model is sensitive to parameters related to the visual and locomotion abilities of larvae to detect and capture the prey. Later, when they establish these abilities, the choice of accepting or not the prey becomes more important.

Résumé : Le taux d'alimentation d'un organisme est contrôlé par des contraintes imposées par les processus associés à la consommation. Notre modèle général d'alimentation incorpore la rencontre de la proie, sa poursuite réussie, sa manipulation et sa digestion dans une seule représentation fonctionnelle dans laquelle la digestion est considérée comme un processus parallèle. Le modèle génère des courbes de réponse fonctionnelle de type II. Toutefois, le taux d'alimentation maximal asymptotique est déterminé par la somme du temps nécessaire à la manipulation et à la digestion d'une proie, moins un gain de temps puisque la digestion est un processus qui se fait en parallèle avec la manipulation. Nous utilisons notre modèle avec d'autres modèles existants de rencontre, de poursuite réussie et de digestion afin d'estimer le taux d'alimentation de larves de poissons. Nous évaluons notre modèle en considérant des données expérimentales obtenues chez des larves de la brême de mer (*Sparus aurata*) et nous trouvons une très forte correspondance entre nos prédictions et les résultats expérimentaux. Une analyse de sensibilité montre que, chez les premiers stades, le modèle est sensible aux variables reliées aux capacités visuelles et locomotrices impliquées dans la détection et la capture des proies par les larves. Plus tard, lorsque ces capacités sont bien établies, c'est le choix d'accepter ou de refuser la proie qui devient plus important.

[Traduit par la Rédaction]

Introduction

The ability of fish larvae to grow and develop in relation to food availability is of prime interest both in natural and in reared fish populations. The larval stage is regarded as the most critical phase of marine fish. Growth and survival depend, to a great extent, on food availability and environmental conditions. During the early developmental stages, feeding is affecting significantly the survival of the larvae and thus their natural recruitment as well as the success of any aquaculture application (Hunter 1980; Houde and Zastrow 1993; Parra and Yufera 2000). The food consumption rate and assimilation efficiency are also determining parameters for establishing successful methodologies of larval rearing for aquaculture purposes. The consumption rate depends on food availability and the developmental stage of the individual (Houde and Schekter 1980; Kentouri 1985), while assimilation efficiency depends on the quality of diet (Houde 1996) and the development of the digestive system of the larvae (Sarasquete et al. 1995).

Theoretically, there are numerous feeding representations; the appropriate model is determined by the constraints on consumption processes applicable to the specific individual organism. Jeschke et al. (2002) provided an overview of feeding models published since 1959 including the most important factors incorporated in each model. The relationship between feeding or ingestion rate (i.e., number of prey eaten per individual predator per unit time) and prey density is termed functional response. The most frequently observed functional response, proposed by Holling (1959*a*) as type II, is a hyperbolic function of prey density. The feeding rate increases almost linearly at low prey densities to an asymptote at higher prey densities with a progressively decreasing rate. Limitation of the feeding rate at high prey densities has been attributed to either handling or digestion constraints. There

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are a number of models that include only handling time or only satiation and considerably fewer models that include both handling time and satiation. Models that incorporate satiation usually quantify it as an abstract variable. The physiological bases are not fully understood and involve glucose levels in the blood (Schmidt-Nielsen 1990; Engstrom et al. 2003). This fast-changing variable is controlled by the digestion process. We therefore directly link the digestion process to the feeding process.

In this paper, we develop a model assuming that searching, encountering, pursuing, attacking, and handling are implemented sequentially, while digestion is executed parallel to the above as a background process. We will call the sequential processes the consumption process. Henson and Hallam (1995) proposed a similar process for feeding, arriving at a feeding rate representation defined as the minimum of the consumption and digestion process. Models with switches are almost always difficult to analyse. Especially in situations where food density changes with time, this representation of the feeding rate can become rather cumbersome. We assume that the time periods required to perform the different tasks are random variables following specific distributions and arrive at a functional response that includes all processes in one feeding representation, thus avoiding the switches.

General models fail to predict the consumption rate for small larval fish. This is because at early stages, larvae have neither developed their abilities for swimming and hunting (Kentouri and Divanach 1982) nor adapted to exogenous feeding (Kentouri and Divanach 1982; Yufera et al. 1993). For the purposes of larval fish feeding, we combine the model with existing models for encounter, successful pursuit, and digestion, applicable to larval fish.

Theoretical development

Sequential processes

The consumption process involves various tasks that occur sequentially, that is, the consumer must complete all tasks before turning attention to another prey. We assume that the prey is randomly distributed and the predator encounters prey according to a Poisson process with a rate R_e. Given an encounter, the predator must successfully pursue and attack the prey and subsequently eat it. We subdivide the consumption cycle into two stages that exclude each other: the pursuit–attack (pursuit stage) and the handling stage. The successful pursuit–attack occurs with probability ρ if the predator does not handle a prey. During handling, no prey is pursued or attacked by the predator, so the success probability for each encountering prey follows a renewal process alternating between the values ρ and 0, when the predator is pursuing and handling, respectively (Nelson 1995; Kooijman 2000).

Let T_p and T_h be the durations of the pursuit and handling stages, respectively. The pursuit period is an exponentially distributed random variable with mean $R_p^{-1} = (\rho R_e)^{-1}$. In some cases, the predator may not eat the particular prey with some probability $1 - \epsilon$. The corrected handling time thus becomes $(\epsilon R_h)^{-1}$, where R_h denotes the handling rate. The cycle period of the consumption process is $T_c = T_p + T_h$ and the inverse of its expected value, $R_c = 1/\mathrm{E}T_c$, equals the mean consumption rate; it is defined as the ratio of the cumulative number of events occurring in a large period to the length of this period. Consequently

$$(1) \qquad R_c = \frac{1}{(\rho R_e)^{-1} + (\epsilon R_h)^{-1}}$$

The successful completion of the sequential processes consists of the subcomponents: (*i*) encounter rate, (*ii*) probability of successful pursuit–attack, and (*iii*) probability of eating.

Parallel processes

The second step in deriving the feeding rate is to model the independent parallel processes of consumption and digestion. Let the consumption and digestion periods T_c and T_d be exponentially distributed random variables with means R_c^{-1} and R_d^{-1}, respectively. We assume that feeding is constrained by the minimum process rate or else by the maximum process time. Thus, the feeding time of one prey is $T_f = \max\{T_c, T_d\}$. The distribution function of T_f equals the product of the distribution functions of the independent random variables T_c and T_d:

$$\Phi_{T_f}(t) = [1 - \exp(R_c t)][1 - \exp(R_d t)]$$

The expected value of the feeding period is

$$\mathrm{E}T_f = \int_0^\infty (1 - \Phi_{T_f}(t))\,\mathrm{d}t = R_c^{-1} + R_d^{-1} - (R_c + R_d)^{-1}$$

The inverse of its expected value, $R_f = 1/\mathrm{E}T_f$, is the mean feeding rate; it is defined as the ratio of the cumulative number of prey eaten in a large period to the length of the period. Therefore, the mean feeding is

$$(2) \qquad R_f = \frac{1}{R_c^{-1} + R_d^{-1} - (R_c + R_d)^{-1}}$$

Equation 2 with eq. 1 gives the feeding rate when digestion is modeled as a parallel process:

$$(3) \qquad R_f = [(\rho R_e)^{-1} + (\epsilon R_h)^{-1} + R_d^{-1} - [[(\rho R_e)^{-1} + (\epsilon R_h)^{-1}]^{-1} + R_d]^{-1}]^{-1}$$

We will call this feeding representation parallel feeding. Note that the parallel feeding period is equal to the sequential feeding period minus the gain in time.

Larval feeding model

For the larval feeding model, we recognize the following events: larvae must encounter a prey, attack the prey, capture the prey, and successfully ingest the prey. If there is no encounter, there can be no feeding. Hence, the rate of encounter will directly affect the amount of food a larva eats. Encounter is necessary but not sufficient condition for consumption; the postencounter components of ingestion, pursuit, attack, capture, and eating, as well as the digestion play an important role. To obtain an accurate assessment of the feeding rate at early developmental stages, we must quantify the subcomponents of the feeding cycle. The most frequently used symbols are presented in Table 1.

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Table 1. Frequently used symbols.

Symbol	Dimensions	Interpretation
R_f, R_e	No.·t^{-1}	Rates of feeding (f) and encounter (e)
R_h, R_d	No.·t^{-1}	Rates of handling (h) and digestion (d)
X	No.·l^{-3}	Prey density
E	l^3·t^{-1}	Encounter rate coefficient
ρ	—	Probability of successful pursuit
ϵ	—	Probability of eating
r	l	Encounter radius
α	rad	Encounter angle
u	l·t^{-1}	Mean velocity of the prey
v	l·t^{-1}	Mean velocity of the predator
k	t^{-1}	Gut clearance rate coefficient

Note: In the dimensions column, l is length and t is time.

Encounter rate

Organisms use many methods to obtain their food; some sit and wait for their prey to pass by, while others search actively. As feeding methods are species specific, the encounter rate can be calculated by various formulas from different authors (Gerritsen and Strickler 1977; Giguère et al. 1982). For a three-dimensional model, e.g., in aquatic systems, one may assume a spherical encounter volume (Gerritsen and Strickler 1977) or a cylindrical encounter volume (Giguère et al. 1982).

For the purpose of this model, we follow Gerritsen and Strickler's (1977) approach. The assumptions of their model are as follows: (*i*) the animals are considered dimensionless points in space, (*ii*) the animals are randomly distributed, (*iii*) the animals are swimming in random directions with a uniform probability distribution, (*iv*) the mean swimming speed of each species will be used in the derivation, and (*v*) predators have an encounter radius, r, that is assumed to be constant in all directions. The last assumption results in the so-called spherical encounter volume. For the purpose of this model for the larval fish, we modified the last assumption to a conical encounter volume. Fish larvae at early stages do not have the ability to sense prey in all directions (Kentouri and Divanach 1982; Browman and O'Brien 1992); they limit their reactive field to a forward-directed conical volume of radius r and angle $\alpha \in (0, \pi/2)$. This assumption modifies Gerritsen and Strickler's (1977) eq. 5 to

$$R_e = EX \tag{4}$$

where X is the density of the prey and E is the encounter rate coefficient that represents the volume swept per unit time by the searching larval and is given by

$$E = \frac{\pi r^2 \sin^2\alpha}{6uv}\left[(u^2 + v^2 - 2uv\cos\alpha)^{3/2} - |u - v|^3\right] \tag{5}$$

where u and v are the mean velocities of the prey and predator, respectively.

The predator's encounter radius r is a complex function of many variables (Gerritsen and Strickler 1977; Scharf et al. 2003). Reaction distance is an important behavioral parameter controlling the feeding process that depends on the visual sensitivity of the larva as well as the prey image area and the ambient light (Aksnes and Utne 1997).

Probability of successful pursuit–attack

Direct observations show that before making the final attack, larvae require time to orient and maneuver themselves into attack position once they detected a prey (Browman and O'Brien 1992). During that time (pursuit time) prey might move out of the larva's sight. Thus, the probability of successful pursuit given an encounter depends on the movement of the prey. During pursuing, larvae attempt to keep prey directly in front of their snout by making slight adjustments in orientation using the pectoral fins (Browman and O'Brien 1992). Thus, the encounter of a prey may result in successful pursuit only if the prey will remain within encounter distance r from the larva, i.e., within the larval encounter sphere, for at least the minimum pursuit time t_p. The distance that the prey can move in the minimum pursuit time t_p defines the prey excursion sphere.

MacKenzie et al. (1994) developed a model that we will use in the present study to evaluate the effects of turbulence on the probability of successful pursuit. The model assumes that the probability of successful pursuit, $P(\mathrm{sp})$, is the ratio of the volume of overlap between the prey excursion sphere and the larval encounter sphere over the prey excursion sphere. The resulting equation (eq. 6 in MacKenzie et al. 1994) is

$$P(\mathrm{sp}) = -\frac{3}{4}\frac{a}{\omega t_p}\left[\frac{r^2 - (\omega t_p)^2 + a^2}{2a\omega t_p}\right]^2 - \frac{3}{4}\frac{a}{\omega t_p} + \frac{1}{4}\left[\frac{2r^3}{(\omega t_p)^3} + 2 + \frac{a^3}{(\omega t_p)^3}\right]^2 \tag{6}$$

where ω is the velocity of the water currents and a is the distance between the larva and the prey item with the constraint that $a \le r$. Under this constraint, eq. (6) has three valid domains ($\omega t_p < r$, $r < \omega t_p < 2r$, and $\omega t_p > 2r$). Integration of eq. 6 over the three valid domains gives the expected $P(\mathrm{sp})$:

$$\overline{P(\mathrm{sp})_{\omega t_p < r}} = \frac{1}{r}\left(\int_0^{r-\omega t_p} 1\,\mathrm{d}a + \int_{r-\omega t_p}^{r} P(\mathrm{sp})\,\mathrm{d}a\right) \tag{7a}$$

$$\overline{P(\mathrm{sp})_{r < \omega t_p < 2r}} = \frac{1}{r}\left(\int_0^{\omega t_p - r} \frac{r^3}{(\omega t_p)^3}\,\mathrm{d}a + \int_{\omega t_p - r}^{r} P(\mathrm{sp})\,\mathrm{d}a\right) \tag{7b}$$

$$\overline{P(\mathrm{sp})_{\omega t_p > 2r}} = \frac{1}{r}\left(\int_0^{r} \frac{r^3}{(\omega t_p)^3}\,\mathrm{d}a\right) \tag{7c}$$

The probability of capture (attack success) can be assumed to be constant, since the final attack takes place only when the prey is separated from the predator by a very short distance and after the predator has maneuvered into position so that the prey is focused within its field of view (MacKenzie et al. 1994).

Probability of eating

Observation of larvae under rearing conditions showed that in several cases, especially during the first days of feeding, individuals after a successful attack frequently abandon

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(regurgitate) the prey. This behavior, which is species specific, depends also, to an extent, on the type of prey (Kentouri and Divanach 1982; Megrey and Hinckley 2001). It is related to the adaptation of larvae to exogenous food and it is also expressed when a change of food occurs in rearing conditions. The probability from 30% to 50% the first day of feeding increases to 100% within a period of a few days.

Digestion rate

The process of digestion is a coordinated combination of physical, chemical, and enzymatic activities that begin as soon as food is taken into the mouth and ends when feces are excreted (Rust 2002). These processes take place in different digestive organs, including the stomach and gut. We do not model the digestion process in detail but are only interested in the rate of food evacuation (gastric emptying rate).

If the organism keeps its gut filled to its maximum capacity, M_{gm}, which can be the case for larvae fish in rearing conditions, this gives the mean food evacuation rate (mean number of prey items that can be cleared per unit time):

$$R_e = k\frac{M_{gm}}{m_p} \quad (8)$$

where m_p is the mean mass of the prey and k is the food evacuation (gut clearance) rate coefficient independent of food density in the environment. This coefficient depends on several factors; the most significant of these are the organism's size, temperature, and prey characteristics such as chemical composition, size, and freshness (Jobling 1981; Knutsen and Salvanes 1999). Since in this formulation of the model, we do not consider temperature variations, we take k to depend only on larval size and type of prey.

Determining the rate of food evacuation has been the objective of many experimental investigations (e.g., see Jobling (1981) for a review). Many studies have demonstrated that at a given temperature and for a given type and size of food, the rate of food evacuation depends on the amount of food remaining in the stomach, while others have shown that it is proportional to the initial gut content.

Assuming complete mixing of food particles in the gut implies first-order kinetics for the change in the amount of food in the gut, M_g, given by

$$\frac{dM_g}{dt} = -kM_g \quad (9)$$

Many empirical data on gut clearance can be fitted to the exponential (or volume) model 9, while some other gut clearance data are better described by a surface area model (Jobling 1981; Knutsen and Salvanes 1999). The rationale behind the surface area model is that, since the digestive enzymes attack the outer surface of the food, it might be expected that the rate of digestion would be proportional to the food particle surface area. Another approach that has been used to fit gut evacuation data is the linear model, where the gut evacuation rate remains constant and proportional to the initial gut content. The linear model is conceptually identical to the plug-flow reactor and is appropriate in situations where the entire gastrointestinal tract is examined instead of the stomach only (Bochdansky and Deibel 2001).

Table 2. Parameter values for the sea bream larvae model used in the numerical simulations.

Symbol	Interpretation	Value
w	Water current velocity	5.5 mm·s^{-1}
r_a	Reaction distance to length constant	1.0
t_p	Minimum pursuit time	1.7 s
R_h	Handling rate	0.1 prey·s^{-1}
ϵ	Probability of eating	0.8
m_r	Mass of rotifer	0.487×10^{-3} mg C
m_{a_1}	Mass of *Artemia* instar I	2.029×10^{-3} mg C
m_{a_2}	Mass of *Artemia* instar II	2.179×10^{-3} mg C
V_r	Volume of rotifer	1.492×10^{-3} mm^3
V_{a_1}	Volume of *Artemia* instar I	7.330×10^{-3} mm^3
V_{a_2}	Volume of *Artemia* instar II	9.425×10^{-3} mm^3
d_r	Density of rotifer	0.326 mg C·mm^{-3}
d_{a_1}	Density of *Artemia* instar I	0.277 mg C·mm^{-3}
d_{a_2}	Density of *Artemia* instar II	0.231 mg C·mm^{-3}

Testing the model

Test data set

We use three data sets against which to test our feeding model. These data were derived from experimental rearing of sea bream (*Sparus aurata*) larvae. The experiments were conducted at the intensive hatchery of the aquaculture facilities of the Institute of Aquaculture, Hellenic Center for Marine Science, Greece. Larvae rearing was performed at constant temperature (19 ± 1 °C) under two photophases (24 and 18 h). These reflect a difference in the period of feeding activity. Two data sets (E1-24 and E2-24) come from experiments performed under a 24-h photophase in different time periods; the third data set (E3-18) was the result of an experiment performed under an 18-h photophase. Experiments E2-24 and E3-18 were performed in duplicate and experiment E1-24 was performed in five replicates. The actual experimental conditions were established 4 days posthatching, when larvae began exogenous feeding. Larvae were successively fed on rotifer until they reached 5 mm in length, a mixed diet of rotifers and instar I *Artemia* when the larvae were between 5 and 6 mm in total length, and rotifers and instar II *Artemia* thereafter until the end of the experiment. The concentrations of rotifers and *Artemia* were 7 and 1 individuals·mL^{-1}, respectively. The feeding rates were expressed as milligrams of carbon per individual larva. The mean carbon content (per individual) for zooplankton is reported in Table 2.

Parameter estimation and model testing

The parameter values used in the model (Table 2; Figs. 1 and 2) were estimates obtained from the literature or from experiments conducted at the aquaculture facilities of the Institute of Aquaculture, Hellenic Center for Marine Science.

The parameter values associated with the cruising velocity of sea bream larvae were estimated using data from Kentouri

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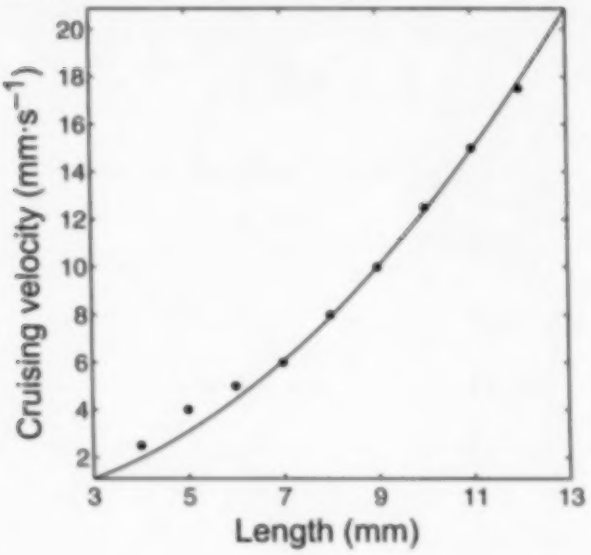

Fig. 1. Larval cruising velocity is proportional to length squared ($v = sL^2$). The parameter estimate (SD) is $s = 0.1235$ (0.0013) mm^{-1}·s^{-1}. Data from Kentouri (1985).

(1985). This data set indicates that larval cruising velocity during early stages increases proportional to length squared (Fig. 1). The velocity of the prey is negligibly small with respect to the velocity of the water currents in the tank and we take it to be equal to the velocity of the currents (i.e., $u = \omega$). The velocity of the currents was not constant during the experiments; it ranged from a minimum value (1 mm·s^{-1}) to a maximum value (10 mm·s^{-1}) (Divanach et al. 1997). Water currents in the rearing tanks are dependent on the recirculation and renewal rate of water. Water movement was almost homogeneous in the rearing tank without stagnant areas. For comparison, Megrey and Hinckley (2001) reported velocity for larger zooplankton to be 0.1–0.45 mm·s^{-1}, thus justifying our approximation that currents control the movement of prey in these conditions.

Reaction distance is an important behavioral parameter controlling the feeding process that is developed during ontogeny (Browman and O'Brien 1992; Scharf et al. 2003). Browman and O'Brien (1992) found that prey location distances increased significantly with fish size for white crappie (*Pomoxis annularis*) larvae. As a first approximation, we assume that the encounter radius increases linearly with the larva's body length L, i.e., $r = r_d L$, where the proportionality constant r_d denotes the larva's encounter distance per unit length. Browman and O'Brien (1992) reported values for the reaction distance-to-length constant almost proportional to length; therefore, we take $r_d = 1$. The encounter angle α depends on the development of the larva's vision system. For the purpose of our testing, it is assumed to be constant and equal to $\pi/6$ (Kentouri and Divanach 1982).

The time t_p required by a larva to take the attack position after encountering a prey is a few seconds. For white crappie, this duration is, on average, 2 s and does not significantly vary with fish size (Browman and O'Brien 1992). Following MacKenzie et al. (1994), we take it to be 1.7 s,

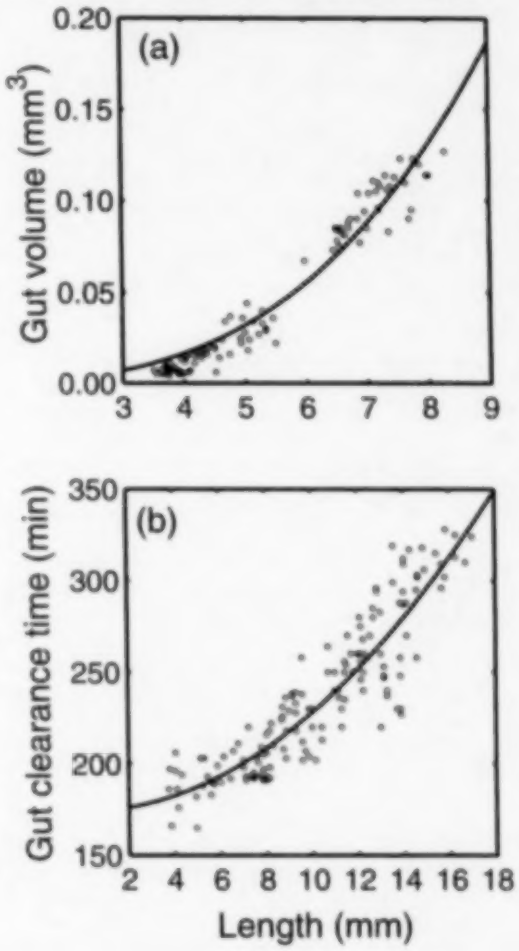

Fig. 2. (*a*) Gut volume is proportional to length cubed ($V_g = cL^3$) and (*b*) gut clearance time is approximated by $T_g = T_0 + bL^2$. The parameter estimates (SD) are $c = 2.56 \times 10^{-4}$ (3.7×10^{-6}), $T_0 = 173.94$ (3.4272) min, and $b = 0.54$ (0.0224) min·mm^{-2}.

while the attack probability is assumed to be 100%. The successful pursuit–attack probability is calculated by one of eqs. 7*a*, 7*b*, and 7*c*.

The handling time of sea bream larvae at the developmental stages under consideration is approximately 10 s·prey^{-1}, ranging from 5 to 15 s (based on our observations). As mentioned previously, the probability of eating varies from 30% to 50% the first day of feeding and increases to 100% within a period of a few days. In our simulations, we consider it to be constant and equal to 80%.

The maximum capacity of the larva's gut, M_{gm}, is proportional to its gut volume, V_g. This means that we can write $M_{gm} = [M_{gm}]V_g$, where $[M_{gm}]$ is some constant that depends on the size of the prey but it is independent of food density and larvae size. When larvae feed on only one type of prey (rotifers), then the proportionality constant is the density of the prey. When larvae feed on both rotifers and *Artemia*

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(instar I or II), then the proportionality constant is computed by

$$(10) \quad [M_{gm}] = \frac{\chi V_r}{\chi V_r + (1-\chi)V_a} d_r + \frac{(1-\chi)V_a}{\chi V_r + (1-\chi)V_a} d_a$$

where d_r, V_r, d_a, and V_a are, respectively, the density and volume of an individual rotifer and *Artemia*, and χ is the proportion of rotifers in the gut. We assume here that the composition of food in the stomach is the same as that in the environment (Eda et al. 1990). For this study, the proportion of rotifer to *Artemia* in the tank is 7:1; thus, $\chi = 7/8$. The shape of an individual rotifer is approximately cylindrical and its volume was calculated assuming a cylinder with mean height 190 μm and mean diameter 100 μm. The shape of an *Artemia* is approximately conical and its volume was calculated assuming a cone with mean height 700 μm and mean diameter 200 μm for instar I *Artemia* and mean height 900 μm and mean diameter 200 μm for instar II *Artemia*.

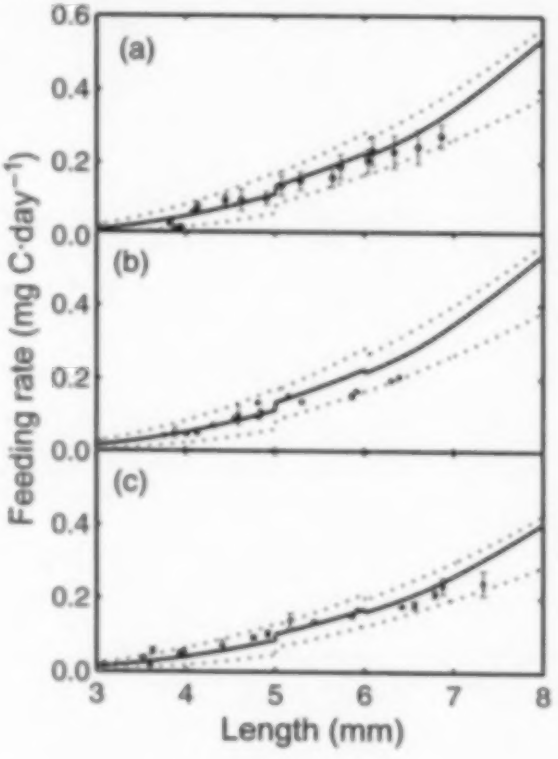
The gut size was measured on 106 fish larvae of size from 3.5 to 8.3 mm and the gut volume was approximated by an ellipse. The gut volume is proportional to cubed length ($V_g = cL^3$) (Fig. 2*a*), and the proportionality constant was estimated to be 2.56×10^{-4}.

The food of larvae consists of planktonic organisms (rotifers and *Artemia*) that, after entering the digestive system, are compressed to form a homogeneous mass, assuming thus complete mixing of food. In this case, the food evacuation rate can be approximated by the model eq. 9. For this model, the gut is never completely emptied, so we specify a percentage p of the original meal and declare the gut empty when only the fraction p of the meal remains. Solving the differential eq. 9, we find that the gut clearance time is $T_g = (\ln p^{-1})/k$; thus, $k = (\ln p^{-1})/T_g$. The gut clearance time T_g was calculated for 150 sea bream larvae of different sizes (Fig. 2*b*). The equation that better describes the data is found to have a quadratic form: $T_g = T_0 + bL^2$. The parameter estimates are $T_0 = 173.94$ min and $b = 0.54$ min·mm^{-2}.

Using the parameter estimates obtained in this section (Table 2; Figs. 1 and 2), we test the parallel feeding model 3 against the experimental data. Because the model measures feeding rate as the number of prey eaten per unit time, while the data are expressed as milligrams of carbon per unit time, eq. 3 was multiplied with the term $\chi m_r + (1 - \chi)m_a$. This term accounts for the different types of prey and represents the mean carbon content of a prey.

A comparison of the predicted feeding rate with the experimental data for each data set is shown in Fig. 3. Because the velocity of the water currents in the tanks was not constant during the experiments, we performed numerical simulations for all possible values in the range 1–10 mm·s^{-1} (Fig. 3, area between the dotted curves). In most cases, the quantitative correspondence between prediction and experiment is remarkably close. The model (with a mean water current velocity) overpredicts the feeding rate for large-sized larvae and, in some cases, underpredicts it for small-sized larvae. This is probably because the velocity of the water currents is initially set at low values and gradually increases to obtain a better circulation of the water. However, almost all of the data fall within the area defined by the dotted curves. For the very few first observations that correspond to small larvae, the model tends to overpredict the feeding rate.

Fig. 3. Feeding rate of an individual sea bream larva as a function of body length. The solid curves are the predictions of the model, with mean water current velocity $w = 5.5$ mm·s^{-1}. The areas defined by the dotted curves represent the model predictions with the velocity of the current ranging from a minimum value of 1 mm·s^{-1} (upper boundary of the area) to a maximum value of 10 mm·s^{-1} (lower boundary of the area). The experimental data sets (given as mean ± SE) are (*a*) E1-24, (*b*) E2-24, and (*c*) E3-18.



This is probably because larvae have not yet developed their ability for swimming and hunting (Kentouri and Divanach 1982) and the minimum pursuit time t_p is greater than 1.7 s; that would have caused a reduction in feeding rate. Another explanation could have been the choice of the gut evacuation model. At this early period (2–3 days after initiation of exogenous feeding), a linear model may be more appropriate, since the stomach is not completely developed and the digestive system of the larva is more like a plug-flow reactor. In this case, the exponential model approach, compared with the linear one, in several cases overestimates feeding rates (Bochdansky and Deibel 2001). The discontinuities in the feeding rate at 5 and 6 mm larval size are due to the introduction of *Artemia* (instar I at 5 mm or instar II at 6 mm) in the larva's diet. The introduction of the mixed diet at 5 mm increases the number of prey available to larvae. The change of prey density, in combination with the different carbon content of each prey type, results in abrupt changes of carbon density, which is reflected by abrupt changes in the feeding rate.

Given that the parameter values of the reaction distance – length coefficient r_a, the minimum pursuit time t_p, and the

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probability to eat the prey ϵ are not well documented for sea bream larvae, we examine the sensitivity of the feeding rate to them. We examine their importance by drawing random variates for each one of them from a triangular distribution. We use this distribution because it has a finite range and it only requires a minimum, a maximum, and a mode. For each of the above parameters, we take the mode to be equal to the value reported in Table 2. The minimum and maximum values are, respectively, 0.4 and 1.2 for r_d, 0.85 and 2.5 for t_p, and 0.2 and 1.0 for ϵ. Similar ranges for these parameters were used by Megrey and Hinckley (2001) for other fish larvae. We generate 500 random variates for each parameter, and for each triplet of the random variates, we simulate the feeding model 3 keeping fixed the rest of the parameters (Table 2; Figs. 1 and 2).

A simulation of the model for a larger range of larvae body length is given in Fig. 4. We find that the variability in the feeding rate neither increases nor decreases monotonically with larval size. Indeed, the coefficient of variation has a minimum at some intermediate mean length (Fig. 4*b*, solid curve). This means that some of the parameters are important for small-sized larvae and others are important for large-sized larvae. It turns out that the most important parameter is the reaction distance – length coefficient, followed by the minimum pursuit time, for the very early developmental stages. The probability of eating the prey has negligible effect. On the contrary, at later developmental stages, the most important parameter is the probability of eating the prey. Furthermore, the reaction distance – length coefficient and the probability of eating the prey are positively correlated with the feeding rate, while the minimum pursuit time is negatively correlated with the feeding rate.

To further examine the effects of water currents on feeding rate of fish larvae, we simulate the deterministic model for larval body lengths beyond our experiments. Although the water currents have an effect on feeding rate at early developmental stages, this effect diminishes as larval size increases (Fig. 5*a*). The effects of the water current velocity on feeding rate for different larval sizes are shown in Fig. 5*b*. These effects over the range of water currents of 1–10 mm·s^{-1} are not monotonous. We observe that for large larval size, the increase in the water current velocity has a negative effect on the feeding rate. The negative effects on feeding rates for large water currents are present also for smaller larvae; however, lower water currents might have negative effects as well.

The resulting relationship between feeding and water current velocity (Fig. 5) is mainly due to the effects of the latter on the encounter rate and probability of successful pursuit. The relationship between the probability of successful pursuit and current velocity (eq. 6) is negative and sigmoidal, while the encounter rate (eq. 4) exhibits a minimum at intermediate values of the current velocity.

Qualitative results

Properties of the parallel feeding equation

The parallel feeding eq. 3 has properties similar to the disc equation. Indeed, substituting eq. 4 into eq. 3, we find that for a certain predator size, the feeding rate is an increasing and concave down function of the prey density X, and its gradient at $X = 0$ is equal to the predator's success rate ρE:

$$\left.\frac{dR_f}{dX}\right|_{X=0} = \rho E$$

As the prey density X approaches infinity, the feeding rate approaches a maximum. The asymptotic maximumfeeding rate is

$$(11) \quad \lim_{X\to\infty} R_f = \frac{1}{(\epsilon R_h)^{-1} + R_d^{-1} - (\epsilon R_h + R_d)^{-1}}$$

Thus, the asymptotic maximum feeding rate is determined by both the handling and the digestion processes. When the digestion process is ignored (i.e., zero digestion time), the

Fig. 4. (*a*) Feeding rate of an individual sea bream larva as a function of body length when the parameters r_d, ϵ, and t_p are drawn from a triangular distribution. The area between the dotted curves give the 95% frequency interval, the solid curve gives the mean, and the dashed curve gives the feeding rate when all of the parameters are constant. (*b*) Coefficient of variation (solid curve). The other curves give the coefficient of variation when all parameters are constant except one at a time: r_d (dashed curve), ϵ (dotted curve), and t_p (dash-dotted curve).

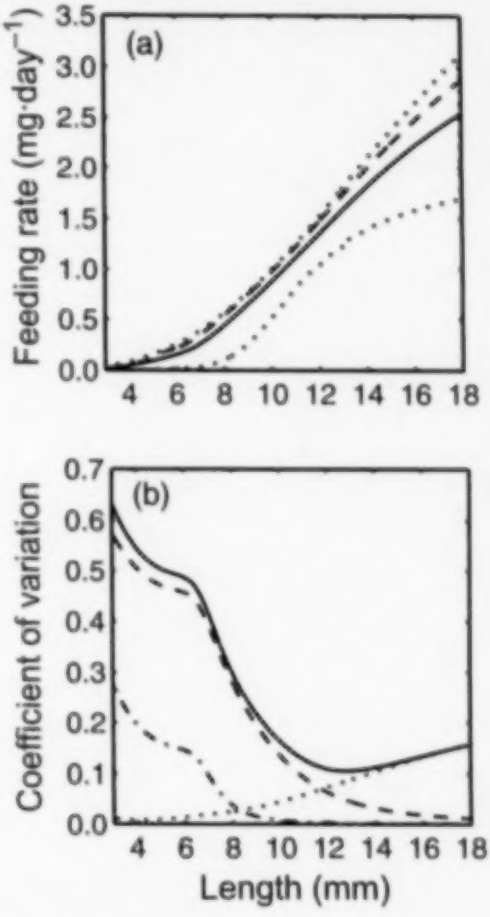

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Fig. 5. (*a*) Feeding rate of an individual sea bream larva as a function of body length. The solid curve is the prediction of the model, with mean water current velocity $w = 5.5$ mm·s^{-1}. The area between the dotted curves represents the model predictions with the velocity of the currents ranging from a minimum value of 1 mm·s^{-1} (upper boundary of the area) to a maximum value of 10 mm·s^{-1} (lower boundary of the area). (*b*) Feeding rate as a function of the water current velocity for different larval body lengths (solid curve, L = 3 mm; dotted curve, L = 4 mm; dash-dotted curve, L = 8 mm). The scaling on the second y axis reflects the feeding rate of a larva with body length L = 8 mm.

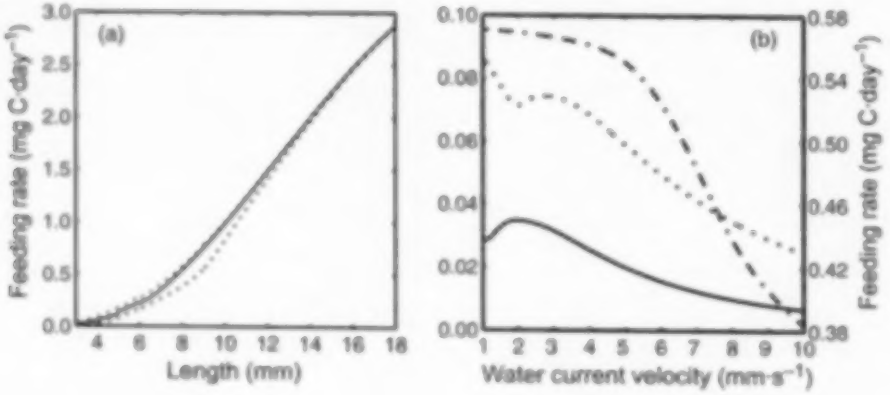
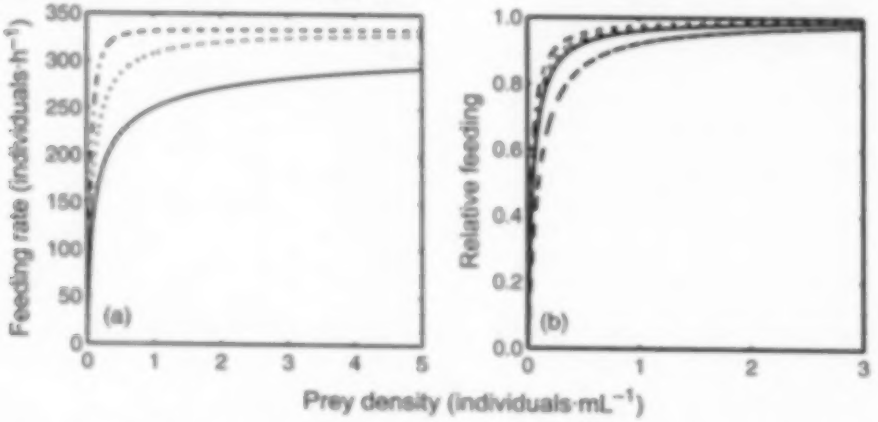


Fig. 6. Feeding rate as a function of prey density. (*a*) The solid curve is the graph of the parallel feeding equation when both handling and digestion times are equally important ($(\alpha R_h)^{-1} = 0.003$ h and $R_d^{-1} = 0.003$ h). The broken curves represent the limiting cases: handling limited (dotted curve) ($(\alpha R_h)^{-1} = 0.003$ h and $R_d^{-1} = 0$ h) and digestion-limited (dash-dotted curve) ($(\alpha R_h)^{-1} = 0$ h and $R_d^{-1} = 0.003$ h). The asymptotic maximum feeding rate for both limiting cases is the same; however, this asymptotic maximum is approached more slowly for handling-limited predators. (*b*) Graphs of the parallel feeding equation (scaled to its maximum) with $\rho = 1$, $(\alpha R_h)^{-1} = 0.003$ h, and $R_d^{-1} = 0.003$ h (solid curve), $\rho = 0.5$, $(\alpha R_h)^{-1} = 0.003$ h, and $R_d^{-1} = 0.003$ h (dashed curve), $\rho = 1$, $(\alpha R_h)^{-1} = 0.005$ h, and $R_d^{-1} = 0.003$ h (dotted curve), and $\rho = 1$, $(\alpha R_h)^{-1} = 0.003$ h, and $R_d^{-1} = 0.005$ h (dash-dotted curve). For all cases, the encounter rate coefficient $E = 4 \times 10^3$ mL·h^{-1}, and where it is not reported, $\rho = 1$.



feeding rate reduces to eq. 1. When the handling process is ignored, the feeding rate is given by eq. 3 by setting the corrected handling time $(\alpha R_h)^{-1}$ equal to zero. The properties of the parallel feeding equation and its limiting cases as a function of the prey density are illustrated in Fig. 6. Note that when both handling and digestion are important, the asymptotic maximum feeding rate is reduced (Fig. 6*a*). When corrected handling time and digestion time are equally important, the asymptotic maximum is equal to $(2/3)R_d$ (or $(2/3)\alpha R_h$).

The rate at which the asymptotic maximum is approached depends on the predators success rate as well as the handling and digestion times (Fig. 6*b*). To compare the slopes of different feeding rate curves, one has to assure that the maximum feeding rates are identical; thus, we scale the feeding rate to its maximum. When time is wasted through unsuccessful pursuits ($\rho < 1$), the slope of the feeding rate curve is reduced. However, when either the digestion time or the corrected handling time increases, the asymptotic maximum decreases (the right-hand side of eq. 11 is a decreasing function of R_d^{-1} and $(\alpha R_h)^{-1}$ and is achieved at a faster rate.

Discussion

We have developed a mechanistic functional response model that treats digestion as a parallel process. The parallel

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feeding model assumes that the digestion process occurs parallel to and independently from the sequential processes of searching, pursuing, attacking, and handling a prey and incorporates all of these processes in one functional representation.

This approach of modeling the parallel feeding processes has its origin in the concept of synthesizing unit (Kooijman 1998, 2000), which in turn has its origins in queueing theory. In its simplest form, the synthesizing unit is an enzyme or a complex of enzymes that binds a substrate molecule to deliver a product molecule or a set of product molecules. The predator can be thought of as a synthesizing unit (or a server) that must complete two tasks in parallel before considering the process of feeding of one prey completed.

Most feeding rate models are built around the Holling's (1959*b*) disc equation, which has the form $\mathcal{E}$(prey eaten/time) = $(T_h + \mathcal{E}T_e)^{-1}$, where T_h is the handling time during which the predator cannot search and $\mathcal{E}T_e$ is the mean time between initiation of search and its termination by a prey encounter event. The shape of the functional response is determined by the relationship between the prey density and predator–prey encounter rate. One way to include both handling and digestion time is to sum them up or to increase handling time (Mills 1982; Henson and Hallam 1995). When modeled this way, it is assumed that the predator cannot search while digesting; all tasks associated with feeding are implemented sequentially. Another way is to combine the disc equation with a digestive capacity constraint. This constraint limits the maximum feeding rate but does not affect otherwise the functional response (Fryxell 1991; Hirakawa 1997; Farnsworth and Illius 1998). Jeschke et al. (2002) developed a simple mechanistic model that treats digestion as a parallel process that influences foraging activity but does not prevent the predator from searching and handling new prey. They let the hunger level determine the probability that the predator searches for new prey. They called the feeding rate equation the steady-state satiation (SSS) equation because of the assumption that the predator's hunger is at steady state.

It is beyond the scope of this paper to compare in detail the parallel feeding with other feeding representations. However, we can briefly discuss some similarities and differences between them. Like other feeding models, the parallel feeding equation produces type II functional response curves. When the parallel feeding equation is compared with existing feeding representations that include digestion explicitly as a sequential process (Mills 1982; Henson and Hallam 1995) or through satiation (the SSS equation (Jeschke et al. 2002)), there are several differences, particularly when digestion and handling times are important. The asymptotic maximum for the parallel and sequential feeding equations depends on both the handling time and the digestion time, while that of the SSS equation is determined by the maximum of the two time periods (Jeschke et al. 2002). The asymptotic maximum of the parallel feeding rate is always larger than the asymptotic maximum of the sequential feeding rate because the consumption and digestion can be executed simultaneously. The maximum is approached almost at the same rate. The asymptotic maximum of the parallel feeding rate is smaller than that of the SSS equation. This is because the SSS maximum is the inverse of only one time period (handling or digestion), while that of the parallel feeding is the inverse of the sum of the two time periods minus the gain in time. If handling and digestion are about equally important, in view of the stochastic nature of feeding processes, it is more likely that both processes will restrict the feeding rate even at high food densities. A further comparison of the two models shows that although the slopes of the feeding curves at zero prey density are equal to the success rate, the maximum of the SSS equation is approached more slowly than in the parallel feeding model. The latter was measured as the ratio of the asymptotic maximum to the half-saturation constant. This difference is mainly because of the way the digestion process influences the overall feeding process. In the SSS equation, the satiation level, which is influenced by digestion, directly determines the probability that the predator searches for new prey and, in turn, it affects the whole consumption process. The direct effect of satiation on the consumption process will reduce the hunger of the predator and therefore its "desire" for feeding. In the case of the parallel feeding model, the digestion and consumption processes are implemented in parallel and the slower of the two determines the feeding rate through a random process. Finally, when either handling or digestion is much more important than the other, then all functional response curves converge. Further comparisons could be possible using extended data of feeding rates for different prey densities and predator species.

Because general models fail to predict the feeding rate for small larvae, we used our model, in combination with existing models of encounter and successful pursuit, to evaluate the feeding rate of fish larvae. We tested the larval feeding model against experimental data for sea bream larvae and found a very close quantitative correspondence between model predictions and experiments. We examined the sensitivity of the model to some model parameters and found that their importance depends mainly on the larva's size. We chose to examine the effects of the reaction distance – length coefficient, the minimum pursuit time, and the probability of eating the prey on the feeding rate. Other parameters as well may affect the feeding rate depending on the larva's size. We have not examined other parameters either because we have had data to support the chosen value of the parameter for sea bream larvae or because the model was the least sensitive to them. For example, the handling time of a prey, R_h^{-1}, was set to 10 s, while observations show that it ranges from 5 to 15 s. Results from our simulations show that the sensitivity of the feeding model to handling time (for larvae up to 18 mm) is similar to that of the probability of eating the prey, ϵ, i.e., the sensitivity of the feeding rate to handling time increases with larval size. Furthermore, the model is less sensitive to handling time compared with the other three parameters. The sensitivity analysis of the three input parameters indicates that for small larvae, the most important parameters are the reaction distance – length coefficient and the minimum pursuit time, while for large larvae, the probability of eating a prey is the controlling parameter. Consequently, these parameters will play an important role in affecting larval growth and survival. Observations confirm (Megrey and Hinckley 2001) that a decrease in pursuit time, as it is being advected through the larvae's visual field, increases growth and survival. Our results also indicate that at early developmental stages, larval feeding and consequently

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growth and survival depend mainly on visual and locomotion abilities to detect and capture the prey. Later, when they establish these abilities, the choice of accepting or not the prey becomes more important. The latter observation could be of interest in both reared and wild larvae as they undergo changes of prey type during their development.

Here, we did not attempt to employ the effects of water current velocity (turbulence) on the model parameters. It is very critical, however, to examine how turbulence affects them, particularly when the model is applied in a natural environment. For example, does the pursuit time increase while the reaction distance decreases in turbulent environments? Results could change if such factors had been included in the model. In the present study, however, we do not expect to have dramatic changes because our experiments are controlled, i.e., the current velocity increases gradually as larvae increase in size.

Previous research (MacKenzie et al. 1994; Megrey and Hinckley 2001) has shown that there is an optimal level of turbulence with respect to the larval feeding rate, leading to a dome-shaped relationship between them (MacKenzie et al. 1994). This means that larval feeding rates are maximal at intermediate levels of turbulence. This is because the encounter rate increases while the probability of successful pursuit decreases with turbulence. Our results support this conclusion for small larvae but not for large larvae. However, highly turbulent conditions will reduce feeding rates below those that occur during calmer conditions, independent of larval size.

The nonmonotonous behavior of the feeding rate as a function of the current velocity for different larval sizes is due mainly to the effects that the current velocity has on the encounter rate. The encounter rate exhibits a variable minimum that occurs at water current velocities equal to the larval velocity. This deviates from previously reported results (Gerritsen and Strickler 1977; Rothchild and Osborn 1988) that present a positive correlation between water currents and encounter rates. The difference in our case is that larval encounter volume is limited to a forward-directed cone instead of a sphere. The calculation of the encounter rate involves the integration of the relative velocity of prey and predator over all possible track angles of the prey. In general, a minimum is expected in the encounter rate when the two velocities have the same norm and direction, and therefore, the relative velocity is zero. By integrating over the encounter sphere, there is no preferential direction, and therefore, there is no minimum. On the contrary, when an encounter cone is considered, then there exists a principal direction associated with a minimum when the two velocities are equal. MacKenzie et al. (1994) recognized the need of assuming a conical rather than a spherical encounter and pursuit volume but did not include it in their model. Note, however, that at later developmental stages, 15–20 days after exogenous feeding depending on species, larvae develop their sensing abilities and the encounter volume becomes a sphere (Kentouri and Divanach 1982).

Although there are many models of larval fish feeding that include several aspects of fish foraging, such as detailed encounter rates and pursuit success (MacKenzie et al. 1994; Fiksen and Folkvord 1999; Megrey and Hinckley 2001), not many studies have modeled digestion explicitly (Knutsen and Salvanes 1999). To the best of our knowledge, this is the first model that includes many aspects of larval foraging (encounter rate, successful pursuit, handling time, and probability of eating a prey) together with digestion. Depending on the developmental stage of larvae and the quality and (or) the quantity of food, any of the above processes may be the limiting factor.

The predictions of the model regarding feeding rates of sea bream larvae are very close to experimental data under various rearing conditions. This is an indication that the model can be used for estimating feeding rates during larval stages that are considered as the bottleneck for any aquaculture operation. In addition, failure or success of recruitment in nature may be linked to several parameters; one of the most important is the feeding requirements, as they control growth (Houde 1996). Other environmental factors affecting larval growth are small-scale turbulence, light, turbidity, and temperature (see Fiksen and Folkvord 1999). These factors can easily be included in our feeding model (e.g., turbulence is already included). Moreover, the model can be incorporated into any individual growth model (e.g., Kooijman 2000; Lika and Nisbet 2000; Nisbet et al. 2000 for a review) so as to address questions such as recruitment success, larval survival, starvation, and recovery in a controlled as well as in a natural environment. These types of models, therefore, are required to explicitly include early fish life stages as a component of a general ecosystem model.

We have confined our model to contain formulations and values for sea bream larvae. However, the parallel feeding model is very general and applicable, following relevant modifications, to other species and developmental stages of fish. Note that the model can be applied not only to aquatic but also to terrestrial species that employ feeding according to the parallel feeding assumptions.

The parallel feeding model is an alternative approach of including both handling and digestion times. The interest in this approach is mainly in its mathematical simplicity and its interesting properties (Kooijman 1998, 2000). Furthermore, the model can be extended to include more than one type of prey without losing its mathematical attractiveness. The extended model will allow the incorporation of preferential feeding and may be used in studies regarding optimal foraging strategies and feeding behavior.

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Comparison of virtual population analysis and statistical kill-at-age analysis for a recreational, kill-dominated fishery

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Abstract: We used simulations to compare the distributions of estimation errors for virtual population analysis using forward calculation (FVPA) and three variants of statistical kill-at-age analysis (KAA). The KAA variants assumed constant, time-blocked, and nonadditive selectivity. Simulations were based on a recreational walleye (*Sander vitreus*) fishery in Lake Mille Lacs, Minnesota. The focus of our experiments was on how model mis-specification (incorrect assumptions about selectivity for KAA or that kill had no error for FVPA) interacted with the magnitude of measurement errors and fishing mortality. We found that KAA models outperformed FVPA when they assumed the correct selectivity pattern, even when kill was measured without error. Of particular concern was a strong tendency by FVPA to overestimate stock size when kill was measured with substantial error. When KAA was based on an incorrect assumption regarding fishery selectivity and kill was measured with little error, wide distributions of errors and substantial biases sometimes resulted. KAA models that allowed fishery selectivity to change over time performed about as well as a constant-selectivity KAA model when selectivity was constant, and they performed much better when selectivity changed over time. Careful consideration of alternative fishery selectivity models should be a fundamental part of any age-structured assessment.

Résumé : Des simulations nous ont permis de comparer les distributions des erreurs d'estimation dans une analyse virtuelle de population utilisant un calcul prospectif (FVPA) et trois variantes d'une analyse statistique KAA (kill-at-age, mortalité à un âge donné). Les variantes de l'analyse KAA présupposent une sélectivité constante, déterminée en fonction du temps et non additive. Nos simulations se basent sur la pêche sportive de dorés (*Sander vitreus*) dans le lac Mille-Lacs, Wisconsin. Nos expériences visent à étudier comment les paramètre erronés des modèles (présuppositions fausses de la sélectivité pour KAA et présupposition d'absence d'erreur de mortalité dans FVPA) interagissent avec l'importance des erreurs de mesure et de la mortalité due à la pêche. Les modèles KAA fonctionnent mieux que les modèles FVPA lorsqu'on présuppose les bons patterns de sélectivité, même quand la mortalité est mesurée sans erreur. Il est particulièrement inquiétant que les modèles FVPA aient une forte tendance à surestimer la taille du stock lorsque la mortalité est estimée avec une erreur importante. Lorsque l'analyse KAA est basée sur des présuppositions fausses concernant la sélectivité de la pêche et que la mortalité est mesurée avec peu d'erreur, il peut en résulter quelquefois de distributions étendues des erreurs et des tendances erronées importantes. Les modèles KAA qui permettent à la sélectivité de la pêche de changer dans le temps fonctionnent presque aussi bien que le modèle KAA à sélectivité constante lorsque la sélectivité est constante et fonctionnent beaucoup mieux lorsque la sélectivité varie dans le temps. Toute évaluation qui tient compte de la structure en âge devrait considérer de façon attentive les différents modèles de rechange de sélectivité de la pêche et en faire une partie essentielle de l'analyse.

[Traduit par la Rédaction]

Introduction

A widely accepted standard for stock assessment is age-structured modeling to integrate information about a fish population (Megrey 1989; Hilborn and Walters 1992; Quinn and Deriso 1999). Kill limits and other biological reference points derived from such assessments form the scientific basis for management of many of the world's most important fisheries. One common modeling family is virtual population analysis (VPA) (Jones 1963; Gulland 1965; Murphy 1965) and its variants: cohort analysis (Pope 1972) and ADAPT (adaptive framework approach to VPA, Gavaris 1988). A second family is statistical age-structured assessment models (Fournier and Archibald 1982; Deriso et al. 1985; Methot 1989). We denote this family as KAA (kill-at-age analysis) to emphasize that it is the number of fish killed, not the number

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caught, that is important for assessment; many fish caught in recreational fisheries may be released unharmed. VPA methods have been popular in eastern North America and Europe, whereas KAA methods have been popular in western North America, South Africa, Australia, and New Zealand; in many other areas, no clear favorite has emerged.

The fundamental difference between the two is that the VPA family assumes that kill-at-age is measured without error. Of course practitioners know that kill is measured with some error, and their use of VPA reflects a belief or hope that results will be robust given the level of measurement error. VPA can use backward or forward recursive calculations to obtain abundance at age. Backward VPA approaches allow for constraints on terminal abundances or fishing mortalities. Such constraints (linkage assumptions) often lack statistical backing or are ad hoc in nature (Quinn and Deriso 1999), but their imposition can increase stability in the estimation routines by limiting the number of parameters that are directly estimated. Both VPA approaches allow year- and age-specific fishing mortality rates to be calculated from the resulting estimates of abundance at age. Earlier VPA implementations amounted to a calculation procedure, not an estimation procedure. Recent VPA implementations statistically incorporate auxiliary data to estimate population size at age, yet they still assume kill is measured without error. Given A ages and Y years of data, there are AY catch-age observations and $A + Y - 1$ year classes present in the database. If, as assumed by VPA, kill is measured without error and natural mortality is known, then estimation of $A + Y - 1$ year-class parameters in a stock assessment model (see Appendix A) is sufficient to determine abundances at all ages. Some implementations of VPA further reduce the number of formal parameters by imposing assumed relationships for terminal fishing mortality rates.

In contrast, if catch is measured with error and natural mortality is known, as assumed by KAA, then estimation of $A + Y - 1$ year-class parameters and AY fishing mortality parameters is necessary to determine abundances at all ages. The total of $AY + A + Y - 1$ parameters is greater than the number of observations, requiring assumptions to reduce the number of parameters so that the model is not overdetermined. KAA reduces the number of fishing mortality parameters that are estimated by assuming separability of fishing mortality into age (selectivity and (or) vulnerability) and year (fishing intensity) factors for a block of years or by assuming some other statistical model for fishing mortality with reduced parameters. Thus, KAA presents concerns about how well selectivity has been modeled and whether results will be robust when the modeled fishing process diverges from the actual one. Even with these selectivity assumptions, KAA requires more formal parameters to determine abundance at age than VPA.

Some researchers believe that VPA is more parsimonious than KAA because fewer formal parameters need to be estimated. Others believe the opposite because the relationships among fishing mortality rates in VPA (without linkage assumptions) are constrained only by the kill data. In this view, each of the unconstrained AY fishing mortality rates increases the effective number of parameters that needs to be estimated. If the kill data can be matched in more than one way, the lack of further constraints on fishing mortality could be a disadvantage for VPA. Furthermore, in the presence of measurement error, VPA can be viewed as an exact solution to a set of catch equations, in which fishing mortality is estimated for each age and year. From this viewpoint, VPA estimates many more parameters than KAA. In contrast, the statistical elegance of KAA may not be reflected in the performance of the method compared with VPA if the specified model for fishing mortality is not a reasonable approximation of the actual processes that are operating.

Although a variety of limitations and assumptions for VPA and KAA are well understood, it is rare to see comparisons of both approaches for a managed fishery. International Council for Exploration of the Sea (1993) and the National Research Council (NRC) (1998) evaluated the performance of several modeling approaches by simulating fish populations and fisheries such that common modeling assumptions would be violated (e.g., ageing error, fisheries catchability changes, kill under-reporting, and age selectivity changes). These studies produced relatively few simulated data sets, with detailed assessments developed by analysts familiar with the different methods. While these studies provided a realistic assessment of how well different analytical approaches and their practitioners could perform on specific simulated data sets, they did not provide general guidance on the applicability of KAA and VPA under a range of different conditions. Punt et al. (2002) compared KAA, several variants of VPA, and several other methods in an extensive simulation study. The model they used to generate simulated data was moderately complex, being spatially, size-, and age-structured, allowed for technological interactions, and was parameterized to reflect assumptions specific to four fisheries. Their generating models did not match the assumptions made by any of the estimating models, as they assumed that total yield was measured without error (although total weight of fish killed had some error because discard was assumed to be measured with error), and their comparisons were based on short (8-year) simulated time series. They found that the KAA estimator generally outperformed VPA, although not always.

The objective of our study was to probe the benefits and shortcomings of several variants of KAA and one implementation of forward VPA (FVPA) in the presence of varying levels of measurement error and fishing mortality. The usefulness and reliability of each approach was tested using simulation studies, in which the assessments were done in an automated and standard way on simulated data sets. Our simulations were based on the recreational-dominated walleye (*Sander vitreus*) fishery of Lake Mille Lacs, Minnesota, and should provide useful guidance for both recreational and commercial fisheries.

We were particularly interested in comparing the relative performance of a VPA method and KAA, when in fact kill was measured with appreciable error. Our intent was to document the trade-offs between the assumption of known catch-at-age in VPA and the selectivity assumptions in KAA. Consequently we sought to minimize other differences between VPA and the KAA methods. We therefore chose a FVPA implementation, which uses a statistical approach and which does not constrain terminal fishing mortality rates through linkage assumptions. We recognize that linkage assumptions can improve estimation by VPA, depending in part on how well the assumed constraints reflect that actual fishing pro-

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cess. However, use of such procedures in our simulations would have confounded our intended comparison by including additional assumptions regarding selectivity in the VPA method. Our use of a statistical FVPA approach also allowed us to handle survey data and estimate survey catchability in the same way as was done by the KAA methods.

Given that KAA replaces the assumption of known kill with assumptions regarding selectivity, simulated data were generated making different selectivity assumptions and our variants of KAA modeled selectivity in different ways. Thus, we also evaluated the performances of KAA assessment models when their selectivity models were misspecified. Our systematic comparison of methods in the face of different levels of measurement error combined with different degrees of model misspecification is a unique contribution of this study.

Methods

We simulated population dynamics, expected kill-at-age, and expected gillnet survey indices of abundance based on our understanding of the recreational-dominated walleye fishery of Lake Mille Lacs. We added measurement error to the expected kill-at-age and age-specific indices of abundance to generate simulated data sets. We fitted a suite of age-structured estimation models to each simulated data set. In generating simulated data, we varied fishing mortality rates, measurement error associated with the kill-at-age data, measurement error associated with the survey, and assumptions about selectivity (and the degree of separability) of the fishery to evaluate how these factors influenced the relative performance of the different estimating models. Equations defining the simulation model are presented in Appendix A, and symbols used in the simulation model and in estimation models that are not defined in the text are given there. Equations in Appendix A are denoted as Ax. Where equivalent quantities are used in the estimation models, as in the simulation model, they are not redefined, and estimated quantities are distinguished from simulated ones by placing a caret over the symbol.

Simulation model

Our generating model assumes a single fishery and single survey with characteristics like those of the recreational fishery and gillnet survey, respectively, on Lake Mille Lacs. Lake Mille Lacs is a 54 000-ha glacial lake in central Minnesota, and among large lakes in the state, it is the most productive for walleye (3.6 kg·ha^{-1}·year^{-1}). Although annual fish quotas have been set to allocate the total allowable kill between state-licensed sport anglers and Ojibwe Indian netters and spearers since 1997, the fishery kill has been dominated by the sport fishery over the time period used in age-structured assessments (Radomski 2003). The sport fishery has been monitored by annual creel surveys since 1983, and estimates of fishery kill-at-age have substantial measurement error (kill coefficient of variation (CV) greater than 10%). Fish populations are also annually monitored with gillnet, electrofishing, and trawl assessment surveys. The gillnet survey provides the longest time series and is the most extensive of these fishery-independent data sources, and relative abundance estimates have substantial measurement error (survey CV greater than 20%).

The population model covers 21 years, from year 1 to year 21, and produces simulated, "actual" abundances at the start of each year for ages 1 through 9 and a 10+ group. Abundance at age-1 (recruitment) in years 2 through 21 and abundance at all ages in the first year took the same values in each simulation, based on estimates of these quantities from age-structured assessment models of the Lake Mille Lacs walleye fishery. Subsequent abundances for ages (a) 2 through 9 and years (y) 2 through 21 follow a standard deterministic mortality model (Appendix A). In our experiments, we considered three scenarios for fishing mortality on fully selected ages (F_y). The status quo pattern was based on fishing mortality rates estimated for the Lake Mille Lacs walleye fishery in past assessments. We also considered two other fishing mortality scenarios, which we think come close to spanning the range that managed walleye fisheries are likely to experience. The low fishing mortality scenario had fully selected fishing mortality set to half of those in the status quo option. The high fishing mortality scenario had status quo rates through year 13 and a linearly increasing fishing mortality on fully selected ages over the subsequent years (Table 1).

We generated data from a model in which fishing mortality was fully separable into year and age effects (i.e., constant selectivity $S_{a,y} = S_a$) and from three models that deviated from full separability (Appendix A and Fig. 1). The first alternative, time-blocked selectivity, was where the selectivity pattern changed every 5 years, but was constant within 5-year time blocks. The next alternative was a nonadditive model with selectivity varying as a function of fishing mortality (Myers and Quinn 2002). Finally, random variability was added each year to a base selectivity pattern. Annual variability was chosen to be larger for younger ages and of a magnitude to create real contrast in annual selectivity (as shown in Fig. 1). The constant selectivity pattern was dome-shaped and based on stock assessment results for the Mille Lacs Lake walleye fishery (Fig. 1). This same selectivity pattern also served as the base pattern for the nonadditive and randomly varying selectivity models and was the selectivity pattern for the first time block in the time-blocked selectivity model. Selectivity for the remaining three blocks was selected to provide contrast in the simulation results, with increased selectivity at most older ages for each succeeding time block.

Observed data for kill-at-age and survey indices-at-age were simulated for years 1 through 20 and years 1 through 21, respectively. Based on modeled abundance and mortality rates, actual numbers killed at age were calculated from the Baranov catch equation (eq. A8). Observed kill-at-age with a median equal to the actual kill was generated by multiplying this kill by a lognormal measurement error term (eq. A9). (Preliminary simulations, using a bias correction factor, set the mean of observed kill equal to actual kill and produced qualitatively similar results.) Gillnet survey indices of abundance were simulated similarly, assuming that the indices were proportional to actual abundance at age, up to a multiplicative lognormal measurement error (eq. A10). The proportionality constants, or catchabilities, were constant over time, but age-specific (Table 1). The kill-at-age and survey

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Table 1. Parameter values used in the simulator.

Year-specific					Age-specific			
Year	Recruitment of age 1	Status quo scenario	Fishing mortality scenario		Age	Population at year 1	Natural mortality rate	Survey catchability
			High	Low				
1	565 088	0.25	0.25	0.12	1	565 088	0.80	2.817×10^{7}
2	5 914 862	0.13	0.13	0.06	2	1 080 982	0.45	1.735×10^{6}
3	273 807	0.28	0.28	0.14	3	127 276	0.24	4.374×10^{6}
4	460 370	0.26	0.26	0.13	4	165 449	0.24	6.751×10^{6}
5	2 093 396	0.44	0.44	0.22	5	1 119 917	0.24	8.493×10^{6}
6	8 863 597	0.23	0.23	0.11	6	148 893	0.24	1.222×10^{5}
7	956 742	0.36	0.36	0.18	7	143 264	0.24	1.277×10^{5}
8	799 512	0.31	0.31	0.15	8	50 421	0.24	1.513×10^{5}
9	3 424 054	0.55	0.55	0.28	9	33 191	0.24	1.589×10^{5}
10	2 402 837	0.41	0.41	0.20	10+	44 814	0.24	1.589×10^{5}
11	2 062 625	0.13	0.13	0.06				
12	1 596 742	0.24	0.24	0.12				
13	1 284 108	0.29	0.29	0.14				
14	1 402 466	0.17	0.34	0.08				
15	478 640	0.21	0.39	0.10				
16	627 093	0.36	0.44	0.18				
17	1 890 010	0.19	0.49	0.09				
18	1 726 248	0.26	0.54	0.13				
19	5 283 640	0.29	0.59	0.14				
20	313 023	0.38	0.64	0.19				
21	1 474 974							

Note: Year-specific parameters included a set of recruitments that were used for every simulation and trajectories of fully selected fishing mortality. Age-specific parameters included the abundance at age in the first year and natural mortality and survey catchability. Selectivity is shown in Fig. 1.

indices of abundance at age errors were generated independently among ages and years, in accord with the assumptions of widely used least-squares fitting approaches.

Estimating models

We developed a single FVPA model and three statistical KAA models using standard methods (Quinn and Deriso 1999). For all the KAA models, the abundances at age in the first year and abundance at age 1 in subsequent years 2 through 21 were estimated as parameters during fitting of the models. The subsequent dynamics of each cohort are described by eqs. A1 through A3, with model-estimated quantities substituted for the true values. In all cases, M was assumed known and took the correct values (Table 1).

In each model, estimated or predicted kill-at-age was calculated from eq. A8 with known quantities replaced by model estimates and predicted survey indices of abundance by $\hat{I}_{a,y} = \hat{q}_a\hat{N}_{a,y}$ (i.e., eq. A10 with model estimates replacing true values and the error term dropped). Survey catchability (q_a) was estimated for ages 1–9, and catchability of the group 10+ was assumed correctly to be equal to the catchability of age 9.

The KAA models differed in how fishing mortality was modeled. The first KAA estimating model assumed constant selectivity over time (eq. A4). The second estimated selectivity in 5-year blocks (eq. A5). The third KAA model included estimation of the one degree of freedom for the nonadditivity parameter (Myers and Quinn 2002). Thus, each of the estimating models matched one of the models used to generate the simulated data. For the constant selectivity model and the time-blocked selectivity model, selectivity at ages 4 and 5 was set to 1, and F_y was estimated for each year 1 through 20. Selectivity for ages other than 4 and 5 was estimated either for the entire assessment period (constant selectivity model) or for each time block (time-block model). For the nonadditive model, eqs. A3 and A6 were reparameterized as suggested by Myers and Quinn (2002) to describe $\ln(F_{a,y})$ by

$$(1) \qquad f_{a,y} = \bar{f} + \bar{f}_y + \bar{s}_a^{1+s\bar{f}_y}$$

so that in addition to γ, $\bar{f}$, $\bar{f}_y$ (constrained to sum to 0), and $\bar{s}_a$ (constrained to sum to 0) were estimated instead of F_y and S_a. The KAA constant selectivity model had 67 parameters (9 parameters for population at age >1 for the first year, 21 parameters for population at age 1, 20 fishing mortality parameters (F_y), 8 selectivity parameters (S_a), and 9 parameters for survey catchability q_a); the time-block model had 91 (9 parameters for population at age >1 for the first year, 21 parameters for population at age 1, 20 fishing mortality parameters, 32 selectivity parameters, and 9 parameters for q_a); and the nonadditive model had 71 parameters (9 parameters for population at age >1 for the first year, 21 parameters for population at age 1, 21 fishing mortality parameters, 11 selectivity parameters, and 9 parameters for q_a).

In each case, the parameters of the KAA models were estimated numerically by minimizing the weighted sum of squares:

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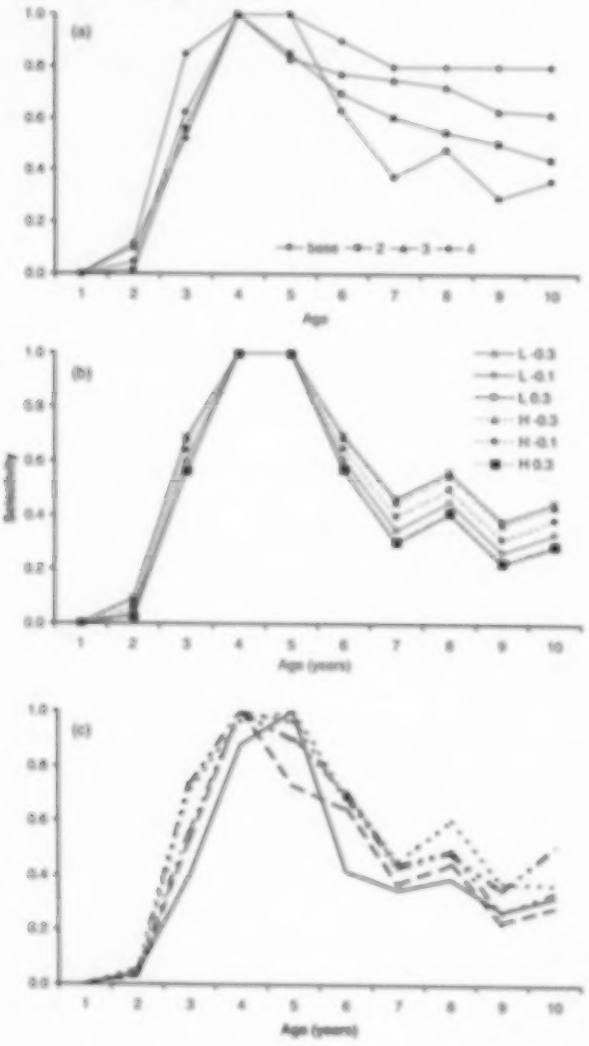
Fig. 1. Fishery selectivity patterns used in generating simulated data. (*a*) Base selectivity pattern C and selectivity patterns (B) used for time blocks two through four. (*b*) Selectivity pattern N in low (L) and high (H) fishing mortality years ($F_y = 1/2\ \bar{f}$ or $= 2\ \bar{f}$) for the nonadditive model with Tukey's nonadditivity parameter (γ) of –0.3, –0.1 and 0.3. (*c*) Five outcomes from the random selectivity model R.



$$\text{RSS} = \text{RSS}(K) + \lambda \text{RSS}(S) \tag{2}$$

$$\text{RSS}(K) = \sum_{a,y} (\ln K^{*}_{a,y} - \ln \hat{K}_{a,y})^2$$

$$\text{RSS}(S) = \sum_{a,y} (\ln f^{*}_{a,y} - \ln \hat{f}_{a,y})^2 \tag{3}$$

where λ is a weighting term and RSS represents the objective function.

For the FVPA estimating model, the initial abundances of each cohort (abundances-at-age in the first year) and subsequent recruitment at age-1 were estimated as parameters. For computational efficiency, Pope's (1972) approximation was used to project subsequent abundances:

$$\hat{N}_{a+1,y+1} = \hat{N}_{a,y} \exp(-M_a) - K^{*}_{a,y} \exp(-M_a/2) \tag{4}$$

and

$$\hat{N}_{10+,y+1} = \hat{N}_{10+,y} \exp(-M_{10+}) - K^{*}_{10+,y} \exp(-M_{10+}/2) + \hat{N}_{9,y} \exp(-M_9) - K^{*}_{9,y} \exp(-M_9/2) \tag{5}$$

As with the KAA methods, the correct natural mortality rates were assumed to be known. The FVPA model had 38 parameters directly estimated (9 parameters for population at age >1 for the first year, 20 parameters for population at age 1, and 9 parameters for q_a). The additional 200 fishing mortality rates were calculated by solving eqs. A1 and A2.

The survey indices of abundance were predicted, as for the KAA models, by $\hat{I}_{a,y} = \hat{q}_a \hat{N}_{a,y}$. As with the KAA models, it was assumed that 10+ age group survey catchability was equal to the catchability of the age 9 group. The parameters (abundance-at-age in the first year, recruitment in subsequent years, and the survey catchabilities (excluding the 10+ group)) were estimated by numerically minimizing RSS(*S*), as expressed in eq. 3. The FVPA estimating model was constrained so that population at age and resulting fishing mortality for age values could only be positive.

The estimations were performed using the Solver routine in Microsoft® Excel (Microsoft Corporation, Redmond, Washington). To assure that the software performed properly, we replicated experiment 1 (see below) using AD Model Builder (Otter Research Ltd., Sidney, British Columbia). We also compared parameter estimates from both software products when presented identical test data sets. Both software products produced similar results, providing partial validation of the simulation results.

Experimental design

For each combination of fishing mortality, survey and kill CVs, and selectivity model, we generated 100 simulated data sets and fitted a suite of assessment models to each data set. Preliminary testing suggested that 100 simulations produced estimates for parameters of error distributions sufficiently reliable for our purposes, and we subsequently verified this by bootstrapping (see "Evaluating the performance of the estimating models"). To evaluate retrospective patterns, we refitted the assessment models to each data set four additional times as the last 4 years of data were deleted sequentially starting with the last year. Unless stated otherwise, the value of λ in eq. 2 for the KAA estimators was set to the ratio of variances of kill and survey (log scale) used to generate simulated data. Thus, we usually assumed that the KAA analyst is aware of the relative magnitudes of variances associated with the kill and survey data. For example, if the kill error had a CV of 5% and the survey error had a CV of 25%, then the weighting on survey residual sum of squares was set at about 0.04 (for a lognormal variable, the log-scale standard deviation is approximately equal to the CV).

Five experiments were conducted. Experiment 1 was intended to guide the design of additional simulation experi-

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ments and provide results for FVPA and KAA models over a wide range of error variances in the data, when the separable fishing mortality model was correct. The design of this experiment was a factorial cross of kill CV (0%, 5%, 25%, 50%) and survey CV (0%, 5%, 25%, 50%). Simulated data were generated using constant kill selectivity following the base pattern and status quo exploitation (Table 1). The value of λ in eq. 2 for the KAA estimator was set to the ratio of variances of kill and survey (log scale), except for when only kill CV = 0% ($\lambda = 0.001$), when only survey CV = 0% ($\lambda = 1000$), and when both kill and survey CV = 0% ($\lambda = 1$). In this experiment, the KAA method was faced only with measurement error. Here (and in our other simulations), FVPA can be viewed as being misspecified except when the actual kill CV was zero, since the assumption of no error in the kill data is incorrect.

Experiment 2 generated data from the constant selectivity model using the base selectivity pattern (Table 1), and thus the KAA method again only faced measurement error. The experiment was intended to explore how fishing mortality might interact with different levels of measurement error to influence the relative performance of KAA and FVPA assessments, when the KAA model was correct. It followed a factorial design that varied fishing mortality (low, status quo, and high), kill CV (5% and 25%), and survey CV (25% and 50%). As in experiment 1, FVPA and the KAA estimator that assumed a constant kill selectivity were applied to each resulting data set. We used the results of this experiment to define a reasonable but more restricted range of these factors for which to evaluate the alternative estimators when the assumption of separability was violated in later experiments.

Experiment 3, our main experiment, was also factorial, including the type of selectivity pattern (time-blocked, nonadditive ($\gamma = -0.1$), and random year-to-year variation), kill CV (5% and 25%), and fishing mortality (status quo and high). Survey CV was 25% in these simulations. All four estimating models were used (FVPA and the three KAA models). Thus, in this experiment the KAA methods were faced with measurement error alone and in combination with model misspecification. Our intent here was to evaluate the relative performance of the different methods when selectivity was no longer constant. We were particularly interested in how various misspecifications of the selectivity pattern would degrade the performance of the different KAA methods and how they would now perform relative to the FVPA.

Experiment 4 was designed to evaluate how more extreme nonadditive fishing mortality than that used in experiment 3 affected assessment performance. The γ parameter, controlling how selectivity varied in response to fishing mortality for the nonadditive model (eq. A6), was set to two values (–0.3, 0.3) and crossed with fishing mortality (status quo and high) in a factorial design. All four estimation models were fitted to each data set, and all simulations used a kill CV of 25% and a survey CV of 25%. An additional level of γ (–0.1) is available from the results of experiment 3 for these same combinations of the other factors. Here, all methods except the nonadditive KAA faced both measurement error and model misspecification.

Experiment 5 evaluated the consequences of setting $\lambda = 1$ in the objective function (eq. 2) for the KAA models, a common approach when variances are not known, rather than to the ratio of the log-scale variances. Simulations were done with the altered objective function, using the time-blocked selectivity models crossed with two levels of measurement error ((*i*) kill CV = 5%, survey CV = 25%, $\lambda = 0.04$ and (*ii*) kill CV = 25%, survey CV = 50%, $\lambda = 0.25$). Because survey CV was greater than kill CV in these simulations, setting $\lambda = 1$ has the effect of increasing the emphasis on the survey data relative to the kill data. A status quo fishing mortality rate was used in these simulations, and the three KAA estimating models were fitted to each data set. Results from experiment 3 provide comparative results for the lower level of measurement error, when λ was set to the ratio of the log-scale variances. Here, all but the time-blocked KAA models were misspecified both for the new simulations and the comparative cases from experiment 3. All the KAA models can be viewed as misspecified for the simulations with $\lambda = 1$, since incorrect relative variances are being assumed.

Evaluating the performance of the estimating models

The purpose of most stock assessments is to generate estimates of population parameters from the most recent time period. We evaluated the performance of the estimators based primarily on the distribution of relative errors in spawning biomass at the start of year 21 and on the distribution of relative errors in exploitation (ages 2 and older) during year 20. For the calculation of spawning biomass, mean weight-at-age and maturity schedules from Lake Mille Lacs were used. Relative error is defined as the percentage r = (estimated – true)/true × 100 for each quantity for a given simulation. In addition to plotting distributions of these relative errors, we also summarized overall performance by the median of the relative error distribution (MRE) and by the median of the absolute values of the relative errors (MARE). We used MRE to indicate median relative bias, the tendency to consistently underestimate or overestimate. We used MARE to summarize the uncertainty or imprecision in the estimates. We used MRE instead of mean relative error and MARE instead of root mean square error because we encountered occasional extreme outliers (positive errors for spawning biomass) that required use of these robust statistics. For all combinations of generating models, estimating models, and error levels represented in our experiments, we calculated bootstrap standard errors (SE) for MARE and MRE for both spawning biomass at the start of year 21 and exploitation during year 20 (based on 1000 bootstrap samples in each case). We examined plots of these SEs versus the absolute values of MRE or versus MARE to gauge our ability to distinguish differences.

In addition, we examined time trends in relative errors for exploitation and spawning biomass and examined retrospective patterns in these variables (Parma 1993), although we present these time trend results only for selected cases.

Results

Uncertainty in estimates of MRE and MARE was evaluated by calculating SEs based on bootstrap sampling. For both spawning biomass at the start of year 21 and exploita-

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Table 2. Comparison of forward virtual population analysis (FVPA) and a statistical kill-at-age model (KAA-C) that assumed constant selectivity over time in experiment 1.

	KAA-C				FVPA			
	Survey CV				Survey CV			
Kill CV	0	5	25	50	0	5	25	50
Spawning biomass MRE								
0	0.00	−0.18	−0.17	−0.21	0.75	0.38	−0.33	1.94
5	0.14	−0.40	−0.09	−1.73	1.40	0.96	0.26	2.02
25	0.36	−0.98	−1.11	0.43	15.76	16.06	15.93	24.18
50	0.61	−0.70	−2.11	0.63	83.34	84.79	102.62	119.53
Spawning biomass MARE								
0	0.00	0.18	0.52	0.96	0.75	1.80	9.67	19.14
5	0.40	1.88	4.55	5.86	1.52	2.35	10.26	19.06
25	2.10	3.43	9.42	14.87	15.76	16.06	17.11	26.95
50	3.93	5.01	10.64	18.82	83.34	84.79	102.62	119.53
Exploitation rate MRE								
0	0.00	0.14	0.14	0.17	−0.75	−0.65	−0.51	−1.39
5	−0.09	0.53	0.20	1.41	−0.96	−1.36	−1.43	−1.37
25	0.14	1.83	2.15	2.77	−7.41	−7.79	−9.89	−13.66
50	0.20	2.16	3.36	2.55	−33.49	−33.89	−38.62	−43.44
Exploitation rate MARE								
0	0.00	0.15	0.41	0.78	0.75	1.49	7.31	15.11
5	0.18	1.71	3.83	5.07	1.79	2.48	8.65	14.86
25	0.78	4.32	8.90	11.57	9.59	11.15	16.04	21.93
50	1.52	6.41	11.33	17.32	33.49	33.89	39.87	44.02

Note: Median relative error (MRE) and median absolute relative error (MARE) of estimates of the most recent spawning biomass and exploitation rate are given as a function of increasing kill and survey error (kill coefficient of variation (CV) = 0%, 5%, 25%,and 50%; survey CV = 0%, 5%, 25%, and 50%) with the status quo exploitation patterns and constant kill selectivity in the simulation model.

tion in year 20, the SEs for MRE were usually well below 5. For exploitation, the maximum estimated SE was 7, which occurred for an absolute MRE of 18. For spawning biomass, SEs exceeded 7.5 in only two cases, taking a value of 16 for an absolute MRE of 20 and an SE of 24 for an absolute MRE of 120. Thus, we focus on differences in MRE of 10 or more or smaller differences representing general patterns in the results. SEs for MARE were more closely tied to the estimated magnitude of MARE, generally being about 10% of the MARE for both spawning biomass and exploitation. Thus, in the case of MARE we focus on differences on the order of 20% of the larger value being compared or general patterns for smaller differences.

Experiment 1 generated data from a purely separable model with constant selectivity, over a range of kill and survey errors, and with the status quo fishing mortality pattern. Not surprisingly, both the FVPA and KAA estimators generally performed better (MRE closer to zero, MARE smaller) when kill and survey CVs were lower (Table 2). The KAA model outperformed FVPA for all combinations of kill and survey CVs (Table 2). The difference in performance was due to both spread of the error distributions and how close the distributions were to being centered on zero. KAA estimates had MRE near zero under all conditions, whereas FVPA had MRE substantially above zero for spawning biomass and substantially below zero for exploitation when kill CV = 25%.

With no error in the kill, both KAA and FVPA were correctly specified, yet under these conditions FVPA still had higher MARE than KAA (Table 2). Not surprisingly, with no measurement error, FVPA had MRE that diverged only slightly from zero (<1 in absolute value) because of the use of Pope's approximation. With no or little (CV = 5%) error in the kill, there is no clear pattern for MRE to increase with increasing survey error. Thus the larger MARE for FVPA than for KAA under these conditions (especially when survey CV = 25%) reflects a broader distribution of errors for FVPA. This can be viewed as a cost incurred by VPA for effectively estimating more quantities than are needed to describe fishing mortality patterns.

Misspecification by FVPA that there is no error in the kill led to even poorer performance relative to KAA when there was substantial error in the kill. At high kill CV (25% and 50%), the FVPA estimator's high positive MRE for spawning biomass is the dominant influence on MARE, although FVPA still had broader distributions of errors than KAA. For FVPA, the movement of the center of the distribution away from zero is the primary cause for the increase in MARE with increasing error in the kill (Table 2); error distributions only became modestly broader (data not shown).

Experiment 2 also generated data from a purely separable model with constant selectivity, but used a range of fishing mortality and two sets of CV values for fishery kill and survey index data (5%, 25% versus 25%, 50%). Results gave

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Table 3. Comparison of forward virtual population analysis (FVPA) and a statistical kill-at-age model (KAA-C) that assumed constant selectivity over time in experiment 2.

	KAA-C			FVPA		
	Fishing mortality			Fishing mortality		
Kill CV, survey CV (%)	Low	Status quo	High	Low	Status quo	High
Spawning biomass MRE						
5, 25	0.44	−0.09	−1.45	1.07	0.26	1.08
5, 50	0.68	−1.73	−1.12	−2.21	2.02	2.45
25, 25	0.67	−1.11	−0.76	23.32	15.93	13.15
25, 50	2.50	0.43	−1.99	19.92	24.18	16.00
Spawning biomass MARE						
5, 25	7.69	4.55	4.55	17.33	10.26	9.88
5, 50	9.62	5.86	6.06	34.45	19.06	17.25
25, 25	16.67	9.42	8.85	28.45	17.11	14.10
25, 50	26.82	14.87	12.72	42.25	26.95	20.84
Exploitation rate MRE						
5, 25	−0.07	0.20	0.47	−0.84	−1.43	−2.71
5, 50	2.78	1.41	0.96	8.74	−1.37	−5.84
25, 25	−1.45	2.15	1.64	−18.62	−9.89	−7.07
25, 50	−1.54	2.77	1.92	−17.79	−13.66	−12.69
Exploitation rate MARE						
5, 25	6.64	3.83	2.78	13.00	8.65	7.10
5, 50	9.52	5.07	4.05	29.22	14.86	12.85
25, 25	15.10	8.90	5.88	24.69	16.04	13.83
25, 50	25.31	11.57	8.78	40.28	21.93	18.84

Note: Median relative error (MRE) and median absolute relative error (MARE) of estimates of the most recent spawning biomass and exploitation rate are given as a function of fishing mortality (low, status quo, and high), kill coefficient of variation (CV) (5% and 25%), and survey CV (25% and 50%). Constant kill selectivity was operative.

the same general picture as was seen in experiment 1 (Table 3). Not surprisingly, MARE decreased with decreases in fishery or survey CV or with increases in fishing mortality. The FVPA estimator always performed less well than the KAA estimator for all levels of fishing mortality and error variance. The magnitude of change in MARE in response to these factors was greater for FVPA than KAA. In addition to the larger MARE, the somewhat surprising tendency for FVPA to have positive relative errors (as indicated, for example, by MRE) in spawning biomass and negative relative errors in exploitation was evident when kill CV was 25% for all levels of fishing. As in experiment 1, the improved performance of FVPA when kill CV was lower was largely because the distributions of relative errors tended to be centered closer to zero.

The main experiment, experiment 3, generated data based on different assumptions about how selectivity varied over time. The type of temporal variation in selectivity had a large influence on the relative performance of the different estimating models, and this interacted strongly with the kill CV (Table 4; Figs. 2 and 3). When selectivity varied over time, following either the nonadditive model or 5-year time blocks, KAA estimating models that made the corresponding assumption about selectivity performed relatively well, in the sense that MRE was near zero and MARE was either the lowest or near the lowest among the estimators that were considered. When the kill CV was 5%, misspecification by the KAA estimators in how selectivity changed over time led some estimators to have strong tendencies to either over- or under-estimate spawning biomass and exploitation rate (Figs. 2*a*, 2*c*, 3*a*, 3*c*). This was most notable for the nonadditive and constant selectivity estimators when actual selectivity varied in 5-year time blocks and also for the constant selectivity estimator when actual selectivity was nonadditive ($\gamma = -0.1$). Differences among the KAA estimators were substantially less pronounced when the kill CV was 25% (e.g., Fig. 3*a* versus Fig. 3*b*), suggesting misspecification of selectivity is of less consequence when kill is measured with more error and that higher error rate is acknowledged.

As in experiments 1 and 2, the FVPA estimator generally produced a broader distribution of relative errors (Figs. 2 and 3) than the KAA estimators. In agreement with the earlier experiments, the FVPA estimator tended to produce positive errors in spawning biomass and negative errors in exploitation when the kill CV was 25% (Figs. 2 and 3). The broad distribution, combined with a tendency to overestimate spawning biomass, caused FVPA to have higher MARE than any of the KAA estimators when kill CV was 25% (Table 4). A notable exception to the broader distribution for errors from FVPA was that all the KAA estimators produced nearly as broad or broader distributions of errors when selectivity varied randomly from year to year and the kill CV was 5%. Under these conditions, the misspecification of selectivity by the KAA models outweighed benefits of their parsimony and allowance for error in the kill. For this situation,

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Table 4. Results from experiment 3 of median absolute relative error (MARE) of estimates of the most recent spawning biomass and exploitation rate.

		Estimating model							
		Kill CV = 5%				Kill CV = 25%			
Fishing mortality	Selectivity	V	C	B	N	V	C	B	N
Spawning biomass MARE									
Status quo	C	10.26	4.55	4.62	5.84	17.11	9.42	9.03	9.63
Status quo	B	8.28	37.25	7.93	34.20	10.34	9.43	8.27	9.07
Status quo	N	10.17	25.86	8.86	6.55	18.79	9.34	9.69	9.99
Status quo	R	10.77	11.26	8.83	17.39	18.16	10.32	9.44	10.01
High	C	9.88	4.55	4.91	5.00	14.10	8.85	8.62	9.76
High	B	6.63	34.34	5.82	33.03	9.96	6.27	7.36	6.97
High	N	9.72	43.63	11.83	5.15	15.17	9.91	9.78	8.78
High	R	10.31	14.13	11.67	15.47	14.90	9.94	10.38	10.50
Exploitation rate MARE									
Status quo	C	8.65	3.83	3.84	4.36	16.04	8.90	8.11	8.66
Status quo	B	6.92	46.58	5.34	43.67	11.76	11.77	5.68	12.07
Status quo	N	8.98	13.61	5.52	4.57	16.72	9.30	8.86	8.03
Status quo	R	9.03	8.47	7.37	13.04	16.83	9.88	9.03	9.64
High	C	7.10	2.78	2.90	3.16	13.83	5.88	6.42	5.91
High	B	5.30	36.91	3.04	36.07	10.52	8.09	5.39	8.68
High	N	7.57	15.72	5.02	3.18	14.50	7.45	6.81	5.99
High	R	6.90	9.13	6.98	9.17	13.85	7.28	7.44	7.74

Note: Forms of the selectivity pattern were time-blocked (B), constant kill selectivity (C), nonadditive with Tukey's nonadditivity parameter (γ) set to –0.1 (N), and random year-to-year variation (R). Kill coefficient of variation (CV) was either 5% or 25%, and survey CV was always 25%. Fishing mortality was either status quo or high. Estimating models were V for forward virtual population analysis, C for the statistical kill-at-age model (KAA) that assumed constant kill selectivity over time, B for KAA that estimated selectivity in 5-year blocks, and N for KAA that included the estimation of the one degree of freedom for nonadditivity parameter.

the resulting MARE for FVPA was comparable to that of the best KAA models. For other cases with kill CV = 5%, FVPA did not always have higher MARE than the KAA estimators because of the substantial divergence of MRE from zero for some of the KAA estimators.

There was no clear best omnibus estimator over the range of generating models and variables considered in experiment 3. When selectivity was in fact constant, both the time-blocked and nonadditive estimators performed as well or nearly as well as the constant selectivity estimator (Table 4). When the CV in kill was 25%, there was little difference in the performance among the KAA estimators, and all were superior to FVPA (Table 4). When the kill CV was 5%, the time-blocked estimator generally had lower MARE than FVPA when selectivity changes were sustained over a period of time (either constant within a time block or gradual responses to nonadditive fishing mortality). FVPA became a competitive estimator only when kill CV was 5% and variations in selectivity were erratic (random) from year to year.

In experiment 4, we further explored the consequences of nonadditive selectivity by generating data from the nonadditive model setting γ to –0.3 or 0.3, instead of –0.1. The distributions of relative errors for the different estimating models depended jointly on the degree of nonadditivity and the variable being considered. Again, the estimator with correctly specified selectivity (nonadditive) performed well, with relatively narrow error distributions centered near zero leading to low MARE (Table 5). The time-blocked selectivity estimator tended to produce narrow distributions of errors, but these tended to be positive when $\gamma < 0$ and negative when $\gamma > 0$. These factors combined so that the time-blocked estimator often had MARE nearly as small as or smaller than the nonadditive estimator. The constant selectivity estimator generally performed relatively poorly (larger MARE) compared with the other KAA estimators. This was particularly evident when $\gamma < 0$ and for spawning biomass. Large values of MARE were largely the result of MRE diverging from zero for this estimator, taking positive and negative values (for spawning biomass) when γ was negative or positive, respectively. Relative errors in FVPA estimates of spawning biomass had a wide distribution, and as in other experiments there was a tendency for positive relative errors. As a consequence, MARE was generally nearly as large or larger for FVPA than for the worst of the KAA estimators (Table 5). Relative performance of the estimators was generally similar for exploitation as for spawning biomass, with the pattern in MRE being reversed (Table 5).

In experiment 5, we used the time-blocked selectivity generator to evaluate the consequences of specifying weights that were not proportional to the inverse of the variances associated with the kill and survey data, but were instead arbitrarily set to 1 for both data sources. The procedures were somewhat robust to the choice of weights when the CVs were 25% and 50% for the kill and survey, respectively. In this case, the estimators had similar MARE for spawning biomass when weighted correctly, and this remained roughly the same when weights were set equal (Table 6). For exploitation rate, the time-blocked estimator (which correctly mod-

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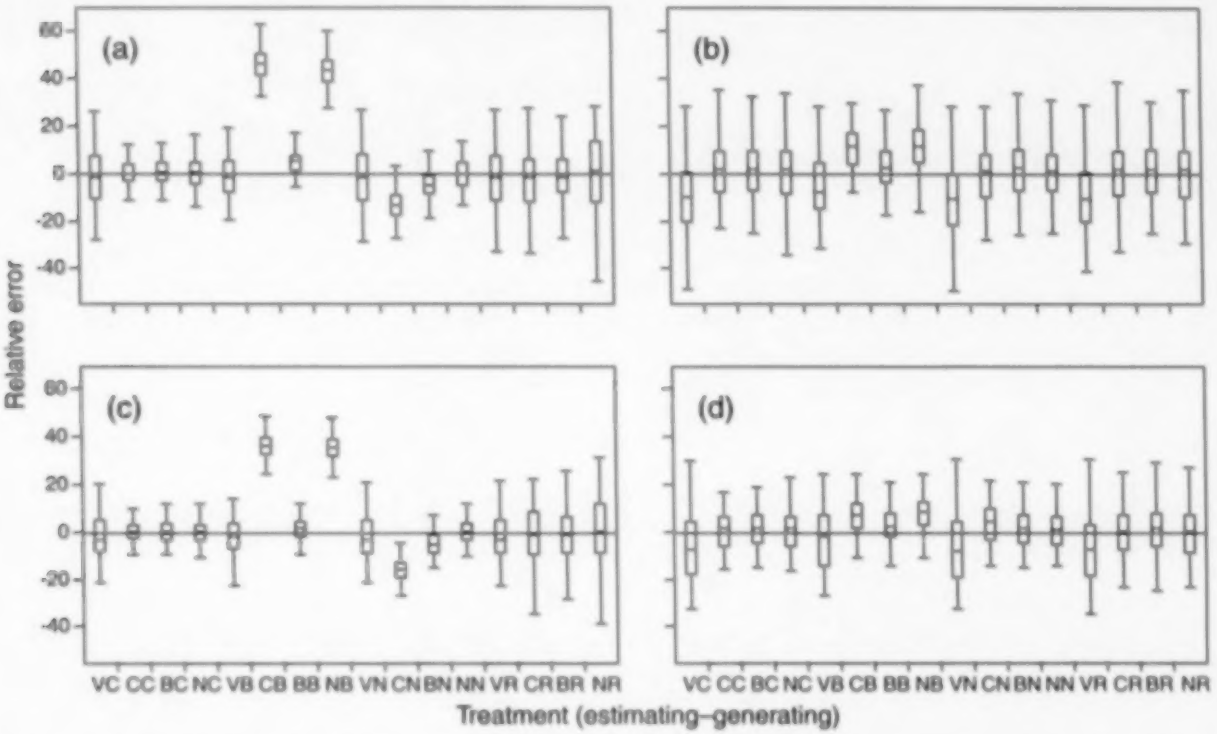
Fig. 2. Box plots of relative error distributions from experiment 3 for exploitation rate in year 20, for (*a*) status quo fishing mortality (*F*) and kill coefficient of variation (CV) of 5%, (*b*) status quo *F* and kill CV of 25%, (*c*) high *F* and kill CV of 5%, and (*d*) high *F* and kill CV of 25%. Boxes denote the interquartile range, and the vertical lines end at the furthest data point not longer than 1.5 times the interquartile range. Treatments are indicated on the *x* axes as *xy*, with *x* taking values of V, C, B, or N and y taking values of C, B, N, and R. *x*, estimating model; *y*, generating model; C, constant selectivity; B, blocks of selectivity; N, nonadditive selectivity; R, random variations in selectivity; V, forward virtual population analysis.



cled selectivity) had lower MARE than the other estimators both with the correct weighting and with equal weighting. However, when data were weighted equally, MARE for this estimator increased, whereas MARE for the other estimators decreased (Table 6). For the case where CVs were 5% and 25% for kill and survey, respectively, there was a much stronger interaction between the weighting and whether the selectivity model was correct or not. For the correct time-blocked estimator, MARE changed little when weighting was changed from correct to equal. However, for the other estimators, when weighting was correct MRE differed greatly from zero, and this difference decreased substantially when weighting was equal (Table 6), leading to smaller MARE. Thus, underweighting kill data actually improved performance of estimators that misspecified selectivity.

Temporal patterns

Heretofore, our evaluation of the relative performance of the different estimating models was based on results obtained for the last year. In general, these results reflected performance over the entire time period modeled. For example, considering experiment 3 (Table 4, Fig. 3), MRE and MARE of spawning biomass for the first year modeled were highly related to the same quantities for the last year (Fig. 4), although there was a general tendency for MRE to be closer to zero in the first year, especially for the most extreme departures from zero. There was a general tendency for the MARE to be less in the first year than in the last year, reflecting tighter distributions of errors in earlier years and MRE closer to zero.

There was a notable exception to the observation that performance of an estimator in the last year was indicative of performance over the time course of the assessment. For the time-blocked generating model, the constant-selectivity estimator produced very strong temporal trends in MRE, illustrated again using spawning biomass from experiment 3 (Fig. 5). Although not illustrated, the nonadditive estimator produced very similar temporal patterns in MREs for this case. The result was strong retrospective patterns. Estimates based on sequentially leaving out years of data starting from the last year produced MREs that increasingly diverged from zero after year 10 (Fig. 6). Interestingly, the magnitude of retrospective errors in Fig. 6 is greater when the kill CV is lower (5% versus 25%), indicating again that greater error in the kill-at-age data ameliorates some of the bias in KAA caused by misspecification of the selectivity model.

Discussion

A general statistical principle is that the accuracy and pre-

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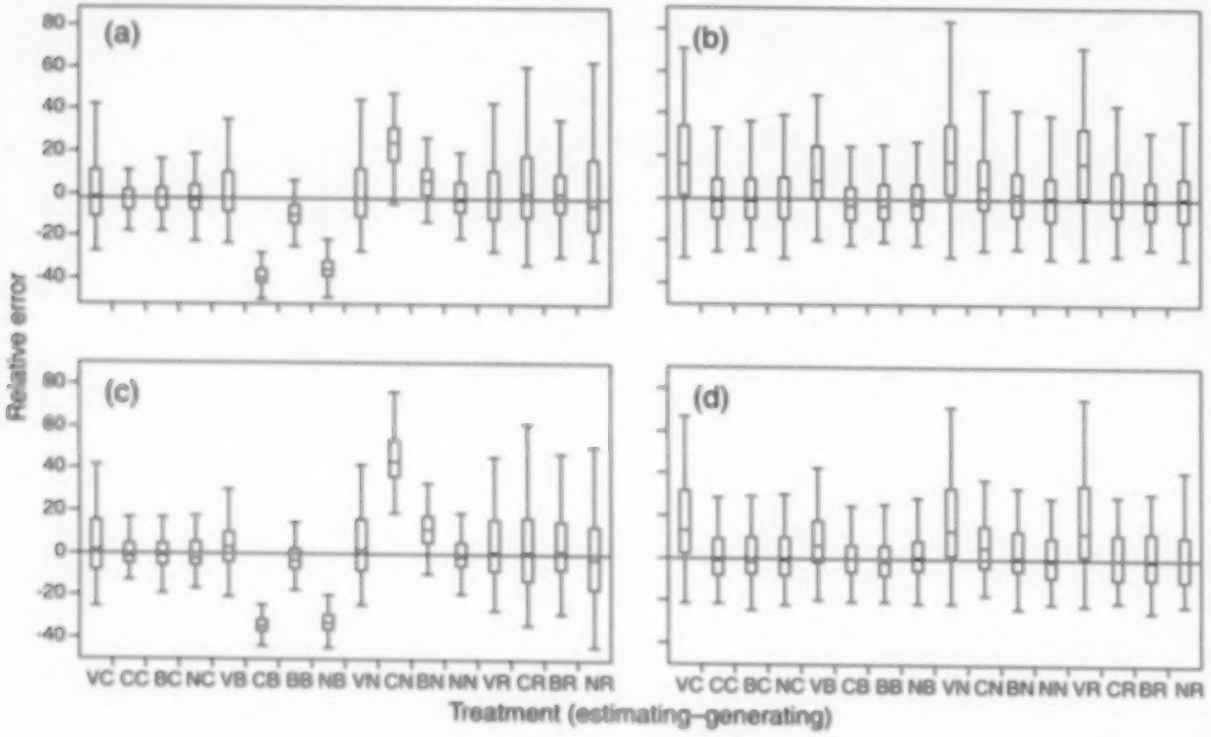
Fig. 3. Box plots of relative error distributions from experiment 3 for spawning biomass at the start of year 21 for (*a*) status quo fishing mortality (F) and kill coefficient of variation (CV) of 5%, (*b*) status quo F and kill CV of 25%, (*c*) high F and kill CV of 5%, and (*d*) high F and kill CV of 25%. Boxes denote the interquartile range, and the vertical lines end at the furthest data point not longer than 1.5 times the interquartile range.



cision in parameter estimation are strongly dependent on the variability in the data sources used: higher input variability should lead to higher output variability. Thus, in this study it is not surprising that increased variability in data inputs often led to increased variability in model outputs, particularly in the absence of model misspecification. In some scenarios, we found that distributions of errors were not centered on zero; in other scenarios, the median error became closer to zero when we added an additional source of model misspecification. Some of these results did not match our a priori expectations. These patterns were related to model misspecification and the interaction of such misspecification with levels of measurement error, which can have somewhat surprising effects in models as complex as fishery catch-at-age stock assessments.

Generally, failure to meet underlying assumptions (model misspecification) led to large median errors in our results. For example, we found high positive median error for population size with the FVPA estimator under high kill CV. This estimator assumed incorrectly that kill-at-age is measured without error. Similarly, we saw that large errors could occur in KAA models when selectivity was misspecified. Interestingly, in the presence of such model misspecification, greater errors could occur with lower data measurement error for the kill. For example, when selectivity was not constant, the constant selectivity estimator had larger error with lower measurement error. This occurred because the RSS(K) had more influence in the objective function with smaller kill measurement errors. In fact, we found in experiment 5 that just specifying an incorrectly higher relative variance for the kill could lead to improved performance when selectivity was misspecified. These results suggest that adjusting assumed relative variances to account for potential model misspecification could improve the reliability of assessments, but this requires accurate perceptions about the potential for such misspecifications. Merritt and Quinn (2000) explored incorporating perceptions into KAA and suggested that one benefit of KAA models is the explicit inclusion of perceptions in a structured framework.

One major concern is the tendency for FVPA to have positive errors in stock size when kill is measured with substantial error. The FVPA method, in an attempt to minimize survey residual sum of squared errors, inflates the number of the youngest age fish to produce a non-negative number of fish in the older age groups. VPA requires that every cohort have enough fish to have supported the total observed removals (plus natural deaths), even if the kill-at-age data contained an outlier due to a substantial error. Such an error can be handled with KAA estimation models without the tendency to overestimate stock size, because they statistically deal with errors in the kill. We believe that VPA estimation models are often incorrectly used where kill is measured with substantial error

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Table 5. Results of experiment 4 with the Tukey's nonadditivity parameter (γ = –0.3, –0.1, or 0.3) controlling how selectivity varied in response to fishing mortality (status quo and high).

	Estimating model							
	Status quo fishing mortality				High fishing mortality			
γ	V	C	B	N	V	C	B	N
Spawning biomass MRE								
–0.3	19.64	22.92	5.64	0.82	14.09	19.75	5.43	0.39
–0.1	17.66	4.97	1.76	0.67	13.26	5.42	0.54	–0.92
0.3	13.30	–14.10	–6.82	–0.33	11.76	–14.19	–7.25	0.28
Spawning biomass MARE								
–0.3	20.28	22.92	10.45	9.50	17.06	19.75	9.92	8.91
–0.1	18.79	9.34	9.69	9.99	15.17	9.91	9.78	8.78
0.3	13.93	14.29	9.44	9.47	12.99	14.66	10.26	8.44
Exploitation rate MRE								
–0.3	–11.00	–6.70	2.05	0.36	–10.49	10.83	1.36	1.12
–0.1	–10.44	0.91	2.70	1.34	–7.49	4.76	2.19	1.55
0.3	–9.03	3.64	–0.20	1.26	–6.79	–7.04	1.05	1.01
Exploitation rate MARE								
–0.3	17.66	11.60	10.87	7.97	14.72	11.43	7.70	6.02
–0.1	16.72	9.30	8.86	8.03	14.50	7.45	6.81	5.99
0.3	13.18	7.69	6.65	9.48	12.65	7.22	5.49	6.56

Note: Median relative error (MRE) and median absolute relative error (MARE) of estimates of the most recent spawning biomass and exploitation rate are given. Kill and survey coefficients of variation (CVs) were 25%. Estimating models were V for forward virtual population analysis, C for the statistical kill-at-age model (KAA) that assumed constant kill selectivity over time, B for KAA that estimated selectivity in 5-year blocks, and N for KAA that included the estimation of the one degree of freedom for nonadditivity parameter.

Table 6. Results of experiment 5 in which the survey weighting in the objective function of statistical kill-at-age model (KAA) estimators was set to either the ratio of kill and survey coefficient of variation (RV) or 1.

	Estimating model					
	Kill CV = 5%; survey CV = 25%			Kill CV = 25%; survey CV = 50%		
Survey weighting	C	B	N	C	B	N
Spawning biomass MRE						
1	–4.47	–3.96	–2.90	–4.19	–5.87	–3.06
RV	–37.25	–7.82	–34.05	–12.91	–5.06	–8.18
Spawning biomass MARE						
1	8.29	7.47	7.97	13.96	13.89	14.64
RV	37.25	7.93	34.20	14.57	13.37	15.31
Exploitation rate MRE						
1	11.25	1.49	12.05	12.70	3.40	13.08
RV	46.58	5.17	43.67	19.36	3.72	17.03
Exploitation rate MARE						
1	11.25	4.71	12.05	13.66	11.19	14.31
RV	46.58	5.34	43.67	19.70	8.87	18.02

Note: The generating model was the time-blocked model. Estimating models are denoted as C for KAA that assumed constant kill selectivity over time, B for KAA that estimated selectivity in 5-year blocks, and N for KAA that included the estimation of the one degree of freedom for nonadditivity parameter. MRE, median relative error; MARE, median absolute relative error; CV, coefficient of variation.

because of concerns about violating KAA kill selectivity assumptions. Our results suggest that KAA estimators are likely to be less erroneous in these situations.

Another widely accepted statistical principle is that overparameterization can lead to poor estimates with high variance (Burnham and Anderson 2002). In our study, we considered two cases particularly relevant to this issue. In experiment 1, both the FVPA and the KAA models correctly described the fishing mortality process in the absence of any error in the kill. While FVPA does not directly estimate more parameters

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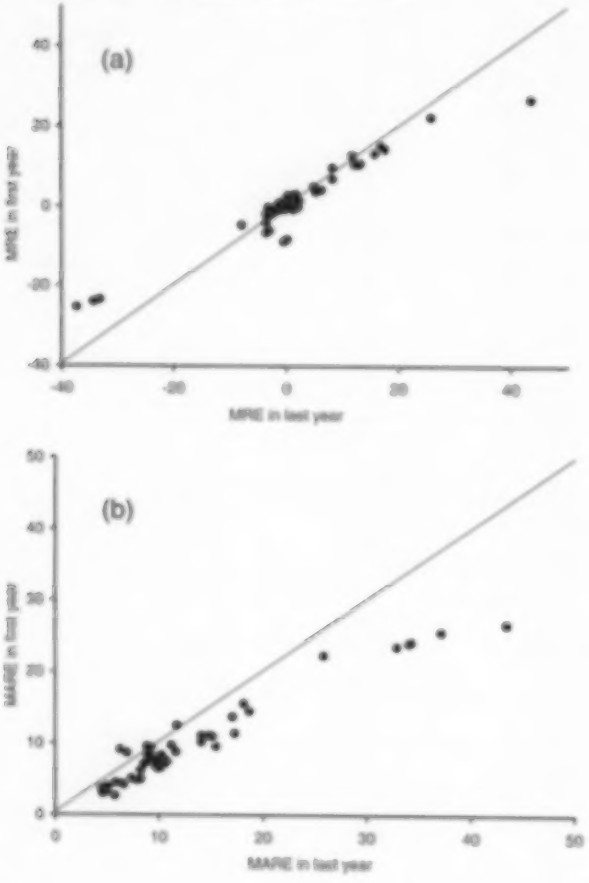
Fig. 4. Results for start of year 1 (first year) versus start of year 21 (last year) of an assessment using all combinations of generating models and estimators for experiment 3. (*a*) Median relative error (MRE) in spawning biomass for the first year of an assessment versus that for the last year of the same assessment. (*b*) Median absolute relative error (MARE) in spawning biomass for the first year of an assessment versus that for the last year of the same assessment. Solid lines are 1:1 lines.



than KAA, we believe it behaves as though it does, because fishing mortality was not constrained to follow the separable model that was correct for this experiment. As a consequence, FVPA performed worse (in terms of higher MARE) than KAA in this experiment, even when kill was measured without error. Interestingly, the higher MAREs for FVPA were not as large as would be expected by adding 200 free parameters for fishing mortality. Nevertheless, the belief in greater parsimony of VPA over KAA by some researchers was not supported by our results.

In experiment 3, one of our generating models assumed constant selectivity. This is correctly modeled by the constant selectivity estimating model and by special cases of the time-blocked and nonadditive estimator. In this case, the additional flexibility in how selectivity was modeled by the time-blocked and nonadditive estimators led to essentially

Fig. 5. Median relative error (MRE) in spawning biomass for each year of the assessments. Results are illustrated from experiment 3 for cases where the generating model used selectivity time blocks and the estimating model assumed constant selectivity. The high fishing mortality scenario is denoted by broken lines and the status quo fishing mortality scenario is denoted with solid lines. Kill coefficient of variation (CV) of 5% is denoted by circles and a kill CV of 25% is denoted by triangles.

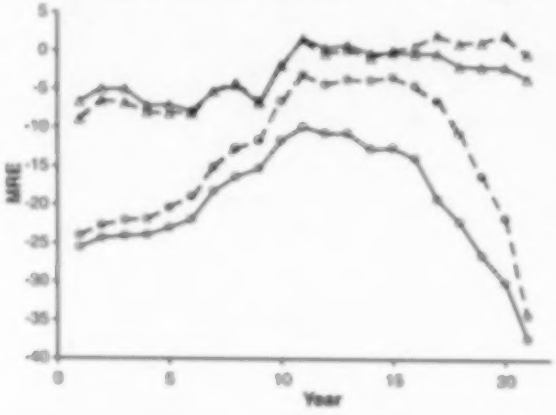
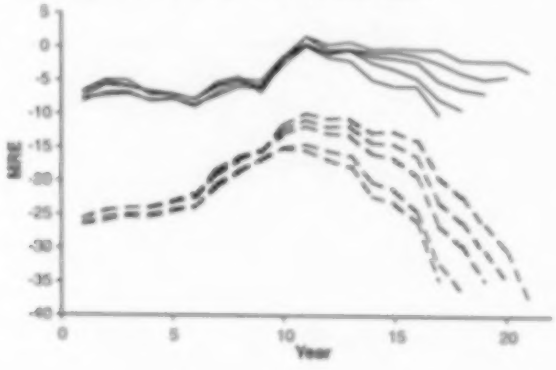


Fig. 6. Retrospective patterns in median relative error (MRE) of spawning biomass. All estimates are based on data generated from experiment 3 for the time-block selectivity model and the status quo fishing mortality rate scenario, with estimates obtained using a constant selectivity model. Kill coefficient of variation was either 25% (solid lines) or 5% (broken lines).



no detectable degradation in the performance of those estimators despite the larger number of estimated parameters.

Three of the generating models for selectivity in experiment 3 had corresponding KAA estimators, so it is not surprising that KAA performed well here. The fourth generating model with random selectivity did not match any of the KAA estimators, so FVPA would be expected to perform better. Indeed, FVPA performed comparably to the time-blocked KAA estimator, better than the nonadditive estimator, and better than the constant estimator under high fishing mortality. It is interesting that FVPA did not outperform all KAA estimators, but perhaps there are selectivity scenarios with even greater interannual variability that would favor FVPA.

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The role of measurement error in the performance of VPA assessments is not entirely clear. Our results indicate that median bias and overall performance of VPA depend upon complex interactions involving details in how measurement errors are generated, other specifics influencing the assessed population and fishery, and approaches used in age-structured stock assessments. Discrepancies in VPA performance in different simulation studies may also be the result of differences in VPA implementation. In contrast with our results, Punt (1997) noted increasing negative bias with increasing measurement errors associated with the kill-at-age composition when evaluating management based on VPA. These biases were not large and he concluded that measurement error in the kill was not a substantial issue for VPA. In his simulations, he first generated lognormal errors for the kill-at-age, then normalized the kill so observed annual total yield matched the true value. We suspect his different result stems from the assumption in his generating models that total yield was without error, an assumption that may be a reasonable approximation for some commercial fisheries, but would not be appropriate for simulating recreational-dominated fisheries. Myers and Cadigan (1995) observed positive bias in maximum likelihood stock size estimates from VPA when survey catch was multivariate lognormal, with the largest bias occurring for the case with the highest survey variance. Punt et al. (2002) reported substantial positive and negative biases for VPA, although at least some of this reflects other differences between the generating and estimating models. They found substantial differences between ADAPT and an ad hoc tuned method, with ADAPT performing more poorly. This result is surprising, as it contradicts the difference in relative performance reported by Patterson and Kirkwood (1995). Punt et al. (2002) suggested the difference might stem from the fact that Patterson and Kirkwood (1995) assumed that the kill-at-age was known without error.

In our study, we chose to use a forward VPA method because this allowed the cleanest comparison of a method that assumed kill was known without placing other constraints on fishing mortality, with methods that acknowledged error in the observed kill but constrained patterns of fishing mortality. Our quantitative results should not be assumed to apply to VPA using backward recursive calculations and constraints on terminal fishing mortality. These backward methods usually include iterative tuning mechanisms, such as setting the fishing mortality for the last age of a cohort to an average for nearby ages in the same year. Differences among implementations in the presence and form of these constraints could be a large part of the reason for some of the discrepancies about the performance of VPA methods described above. If an analyst knew the correct relative fishing mortality rate for older ages, then backwards VPA might perform better. However, KAA performance may improve also if relative selectivities for older ages were known.

Our results with regard to bias of KAA estimators of stock size are consistent with results from other studies. In the absence of model misspecification, median errors (median bias) were near zero, but mean errors (bias) were positive (Bence et al. 1993; Sampson and Yin 1998). Punt et al. (2002) reported both positive and negative biases for KAA, although the negative biases could reflect differences between the generating and estimating models. In developing our simulations, we also explored the use of the nonlinear correction, $\exp(-\sigma^2/2)$, to obtain observed kill-at-age corrected for expected lognormal bias. When we used this correction for the kill and survey data in experiment 1, the KAA estimators produced negative median and mean errors (biases) for population size. Clearly, there remain unresolved issues with regard to bias in age-structured assessments.

NRC (1998) found that model misspecification due to incomplete knowledge accounted for large relative errors in population estimates. Failure to account for changes in selectivity and catchability over time may be the leading cause for poor model performance. Punt et al. (2002) partly attributed the poor performance of all estimating models in their simulation study to the fact that none of them accounted for changes in catchability and each diverged from the generating model in a variety of ways. We found that KAA models that assume no change in kill selectivity when such change was present substantially under- and over-estimated the population, when kill was measured with little error. Butterworth et al. (2003) estimated higher southern bluefin tuna (*Thunnus maccoyii*) spawning biomass with a KAA model that assumed constant kill selectivity than with one without such an assumption. Not accounting for disproportional increases in fishing mortality on less than fully selected ages as fishing mortality increases may lead to overfishing (Myers et al. 1997). We urge caution in applying KAA estimators that assume constant fishery selectivity when selectivity stability is in doubt, especially when kill-at-age observations have low levels of error. In contrast, we found that there was generally little cost in lost efficiency or increased bias by applying models that allowed selectivity to vary over time in a simple fashion, when selectivity was in fact constant. We advocate the use of such alternative models when constant selectivity is in doubt, if only to evaluate the sensitivity of assessment results to the constant selectivity assumption.

There is much work left to be done, both in defining appropriate stock assessment modeling strategies and in determining additional data that could improve assessments. Unfortunately, we did not identify a single simple model that always performed well in the face of different types of temporal change in selectivity, although the KAA estimator that estimated kill selectivity in 5-year blocks was the most robust. Parameter-rich models for time-varying selectivity (for example, models that allow parameters to vary according to random walks (Ianelli 1996; Gudmundsson 1998; Ianelli and Zimmerman 1998)) may perform well under a wider range of conditions. NRC (1998) found such models to be strong contenders. Nevertheless, our results suggest that careful consideration of alternative approaches to modeling time-varying selectivity should become a fundamental part of developing an age-structured assessment. We do not support an approach of avoiding selectivity assumptions by using VPA instead of KAA, except when kill is measured with negligible error and selectivity is likely to vary over time. In our simulations, there was always a KAA variant that performed comparably to FVPA and usually performed much better, even when selectivity varied over time and kill was measured with little error. There are major benefits to

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using KAA if an appropriate selectivity model can be identified.

We strongly urge analysts to consider patterns in residuals, retrospective patterns, and formal model selection criteria (such as Akaike information criterion or for Bayesian applications deviance information criterion) when comparing and evaluating alternatives. The extent to which such efforts can identify correct or at least adequate selectivity models is, however, an important area for future research. Residual patterns in age-structured data should indicate substantial departures from the constant selectivity assumption, but more formal testing is needed. It may be that additional data, such as mark–recapture estimates of absolute stock size or gear studies directed at better understanding age- and size- specific selectivity, will sometimes be required.

Both VPA and KAA modeling approaches make other assumptions we did not investigate, which are also open to question in any particular case (Megrey 1989; Quinn and Deriso 1999). For example, these models usually assume that all fish of a given age (and sometimes age and sex) are equally vulnerable. Both approaches also usually assume that the natural mortality rate is known and often constant, and if the wrong natural mortality rate is used, then these approaches will under- or over-estimate the population size (Lapointe et al. 1992; Mertz and Myers 1997; Clark 1999). Retrospective analysis may indicate when assumptions such as these are violated (Parma 1993; Mohn 1999).

Since the number of comparisons of VPA and KAA models is low, additional work may provide helpful guidance to fisheries managers on the benefits and shortcomings of various modeling approaches. Comparison of our results with those of other researchers suggests that patterns of bias and uncertainty are strongly dependent on the particular situation from which the data and modeling were conducted. Features of our simulation design specific to the Lake Mille Lacs recreational fishery (e.g., fishery selectivity pattern, number of ages, number of years of data) may be responsible for some of the patterns we obtained. Consequently, extensive computer simulation seems essential for validating stock assessments until general principles of statistical properties can be elucidated.

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Appendix A. Model equations used in the simulations of forward virtual population analysis and statistical kill-at-age models.

Population dynamics model

(A1) $N_{a+1,y+1} = N_{a,y} \exp(-M_a - F_{a,y})$

(A2) $N_{10+,y+1} = N_{9,y} \exp(-M_9 - F_{9,y}) + N_{10+,y} \exp(-M_{10+} - F_{10+,y})$

(A3) $F_{a,y} = S_{a,y} F_y$

Alternative selectivity models

Constant selectivity

(A4) $S_{a,y} = S_a$

Time-blocked selectivity

(A5) $S_{a,y} = S_{a,b} \quad \gamma \in \text{block } b$

Nonadditive

(A6) $S_{a,y} = S_a \exp(\gamma \bar{f}_y \tilde{s}_a)$

Randomly varying

(A7) $S_{a,y} = \dfrac{S_a \exp(\varepsilon_{S,a,y})}{\max(S_a \exp(\varepsilon_{S,a,y}))} \quad \varepsilon_S \sim N(0, \sigma_S^2)$

Observation model

(A8) $K_{a,y} = N_{a,y} F_{a,y} (1 - \exp(-M_a - F_{a,y}))/(M_a + F_{a,y})$

(A9) $K^o_{a,y} = K_{a,y} \exp(\varepsilon_{K,a,y}) \quad \varepsilon_K \sim N(0, \sigma_K^2)$

(A10) $I^o_{a,y} = N_{a,y} q_a \exp(\varepsilon_{q,a,y}) \quad \varepsilon_q \sim N(0, \sigma_q^2)$

List of symbols

$F_{a,y}$	fishing mortality rate for age-a fish in year y
F_y	fishing mortality rate in year y on fully selected ($S = 1$) ages
f_y	ln F_y
$\bar{f}$	average over years of f_y
$\tilde{f}_y$	$\tilde{f}_y = f$
$N_{a,y}$	abundance of age-a fish at start of year y
$N_{10+,y}$	abundance of age-10 and older fish (plus group) at start of year y
M_a	natural mortality rate for age-a fish
q_a	survey catchability for age-a fish
$S_{a,y}$	selectivity of age-a fish in year y
S_a	age-specific base selectivity used to determine $S_{a,y}$ for several selectivity models
s_a	ln S_a
$\bar{s}$	average over ages of s_a
$\tilde{s}_a$	$s_a = \bar{s}$
$S_{a,b}$	age-specific selectivity for the 5-year time block b in the time-blocked selectivity model
γ	Tukey's nonadditivity parameter
ε_K	measurement error influencing observed fishery kill-at-age
ε_q	measurement error influencing observed survey index-at-age
ε_S	error causing random variations in selectivity from year to year
σ_K^2	variance associated with measurement error in kill-at-age
σ_q^2	variance associated with measurement error in survey indices-at-age
σ_S^2	variance associated with errors causing random variations in selectivity (0.1 for ages 1–2 and 0.01 for ages 3–10+)

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$S_{max,y}$ normalizing term for random selectivity model equal to maximum value of the numerator of eq. A7 over ages for a year

$K_{a,y}$ kill of age-a fish in year y

$K^*_{a,y}$ observed kill of age-a fish in year y (influenced by measurement error)

$I^*_{a,y}$ observed survey index for age-a fish in year y

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Introduction pathways and establishment rates of invasive aquatic species in Europe

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Abstract: Species invasion is one of the leading mechanisms of global environmental change, particularly in freshwater ecosystems. We used the Food and Agriculture Organization's Database of Invasive Aquatic Species to study invasion rates and to analyze invasion pathways within Europe. Of the 123 aquatic species introduced into six contrasting European countries, the average percentage established is 63%, well above the 5%–20% suggested by Williamson's "tens" rule. The introduction and establishment transitions are independent of each other, and species that became widely established did so because their introduction was attempted in many countries, not because of a better establishment capability. The most frequently introduced aquatic species in Europe are freshwater fishes. We describe clear introduction pathways of aquatic species into Europe and three types of country are observed: "recipient and donor" (large, midlatitude European countries, such as France, the United Kingdom, and Germany, that give and receive the most introductions), "recipient" (most countries, but particularly southern countries, which give few species but receive many), and "neither recipient nor donor" (only two countries). A path analysis showed that the numbers of species given and received are mediated by the size (area) of the country and population density, but not gross domestic product per capita.

Résumé : L'invasion des espèces exotiques constitue l'un des mécanismes principaux de modification des environnements à l'échelle globale, particulièrement dans les écosystèmes d'eau douce. Nous avons utilisé la banque de données DIAS (Database of Invasive Aquatic Species) sur les espèces aquatiques envahissantes de l'Organisation des Nations unies pour l'alimentation et l'agriculture (FAO) pour déterminer les taux de transition des invasions et analyser les voies d'invasion en Europe. Le pourcentage d'établissement des 123 espèces aquatiques introduites dans six pays européens à caractère très différent est en moyenne de 63 %, bien au-delà des 5–20 % suggérés par la règles des dix pour cent de Williamson. Les transitions de l'introduction et de l'établissement sont indépendantes l'une de l'autre et les espèces qui deviennent établies sur une grande échelle le font parce que leur introduction a été tentée dans plusieurs pays et non parce qu'elles ont une meilleure capacité d'établissement. Les espèces aquatiques qui sont le plus souvent introduites en Europe sont les poissons d'eau douce. Nous décrivons des voies précises d'introduction des espèces aquatiques en Europe et nous plaçons les pays en trois typologies, les pays « receveurs et fournisseurs » (des pays européens des latitudes moyennes à grande superficie, tels que la France, le Royaume-Uni et l'Allemagne, qui fournissent et reçoivent la majorité des invasions), les « receveurs » (la plupart des pays, particulièrement ceux du sud, qui fournissent peu d'espèces, mais qui en reçoivent beaucoup) et les pays « qui ne reçoivent ni ne fournissent » d'espèces envahissantes (deux pays seulement). Une analyse des coefficients de direction montre que les nombres d'espèces fournies et reçues sont fonction de la superficie et de la densité de population du pays, mais non du produit national brut par habitant.

[Traduit par la Rédaction]

Introduction

After loss of habitat, invasive species are the second leading cause of biodiversity loss, particularly in freshwater ecosystems (Moyle et al. 1986; Vitousek et al. 1997; Mack et al. 2000). Besides imposing huge economic costs, invasive species cause extinction of native species, reduction of genetic diversity, and biotic homogenization through a variety of mechanisms (Rahel 2000). There are three main stages in the invasion process: the intentional or unintentional introduction to the wild of a species imported into a region beyond its native range; the establishment of self-sustaining, reproductive populations; and population growth and spreading of the species (hereinafter introduction, establishment, and invasion transitions, respectively) (Richardson et al. 2000; Kolar and Lodge 2001). When defining the invasive transition it is better not to consider the ecological or socioeconomic impacts (Richardson et al. 2000; Rejmánek et al. 2002).

Williamson and coauthors (Williamson 1996; Williamson and Fitter 1996) proposed the "tens" rule for invasive species, which states that about 10% (between 5% and 20%) of the species succeed in each of the three transitions (i.e., from

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imported to introduced, from introduced to established, and from established to species with negative socioeconomic effects). Despite the vast literature and great ecological impact of freshwater invasions, the tens rule has been mostly evaluated for terrestrial plants, birds, and biocontrol insects (Williamson 1996; Kolar and Lodge 2001; Lockwood et al. 2001). Although the tens rule is a rule of thumb, there are previous formal tests of the rule (Lockwood et al. 2001), and the exceptions to the rule help to improve our understanding of invasion biology (Williamson 1996). Well-known deviations from the rule are intentionally introduced organisms such as edible crop plants and insects for biological control, which are intentionally introduced and selected by humans to be successful in the receiving ecosystem and, not surprisingly, a higher percentage established (Williamson 1996). Only about 13% of aquatic introductions in Europe are accidental (Welcomme 1992), but it has not been demonstrated whether they deviate from the rule, as would probably be expected.

The two most frequent approaches to the study of invasion biology are identifying species traits that might predict invasion success (Williamson and Fitter 1996; Mack et al. 2000; Kolar and Lodge 2001) and identifying attributes of communities that affect their susceptibility or resistance to invasion (Levine and D'Antonio 1999). In general, the results of these two approaches, mostly based on plants, have often been inconclusive (Williamson 1996, 1999; Smith et al. 1999), and the identification of predominant donor regions and invasion pathways has been recently suggested as a key element to increase the predictive ability of invasion biology (Jenkins 1996; Ricciardi and Rasmussen 1998; MacIsaac et al. 2001), particularly for aquatic invasions (Ricciardi and MacIsaac 2000). This new paradigm seems to be particularly important for taxa such as fishes, whose introductions are mostly not accidental but mediated by human interests. Deliberate introduction implies strong selection of particular taxa and donor and recipient regions, and establishment success should be expected to be higher and less dependent on the features of the species or the receiving ecosystem (Duncan et al. 2003).

Introduction effort, or propagule pressure, is a fundamental but elusive element of this new approach to invasion biology (Williamson 1996; Ricciardi and MacIsaac 2000; Ruiz and Carlton 2003). Unfortunately, in most introductions the number of individuals is often not known (Cassey et al. 2004), and introduction is the least studied of the three invasion transitions despite its alteration being probably the most practical way to mitigate the problem (Kolar and Lodge 2001). The number of species introductions attempted might be a proxy of propagule pressure when this is difficult to measure. Moreover, identification of key donor regions may help to prevent further introductions of new species and to control or eradicate new introductions in the initial phases, when it is more effective (Moody and Mack 1998). Because the geographical distribution of nonmigratory freshwater fish is enormously constrained by watersheds, fish can serve as indicators of introduction pathways for other invasive aquatic taxa that are less known or that expand their distribution range without human intervention.

The objectives of our paper are (*i*) to analyze invasion rates of aquatic species in Europe and compare establishment rates using Williamson's tens rule; and (*ii*) to identify donor regions and invasion pathways within Europe and identify factors that determine which countries act as invasion sources or destinations.

Methods

We used the United Nations Food and Agriculture Organization's Database of Invasive Aquatic Species (DIAS) as the main source of data. DIAS originally considered only freshwater fish species and the records have been published in full by Welcomme (1988) and described without statistical analyses in Welcomme (1991, 1992). The database was later expanded to include other freshwater and marine taxa and nowadays contains about 3200 records (81.9% fishes, 9.4% molluscs, 6.0% crustaceans, and the rest other taxa) that can be searched through the Internet (http://www.fao.org/waicent/faoinfo/fishery/statist/fisoft/dias/mainpage.htm). Of 566 introductions of 113 species in DIAS, 72.5% were intentional, 13% were accidental, and 14.5% occurred for unknown reasons (Welcomme 1991); an updated search of DIAS yielded 3200 introductions, of which 9% were accidental and 17% occurred for unknown reasons. For each species introduction, the database includes, when known, the countries of origin and destination, the year of introduction, whether the species established in the wild, and whether it had ecological effects in the recipient country. From the DIAS database, we made the following changes: "established in the wild through continuous restocking" to "not (naturally) established in the wild"; "probably established" to "established" or similar statements ("unknown" was not changed); we replaced time intervals during the year of introduction with the midpoint; and we did not consider the subspecific level. DIAS records "beneficial" and "adverse" ecological effects, but these qualifications would be controversial and we simply regarded both as ecological effects.

In the DIAS database, three stages were available: introduction, establishment, and occurrence of ecological effects. DIAS clearly underestimates the total number of species introductions, particularly of plants and invertebrates and those not established (e.g., some individuals of many tropical aquarium fish species are probably released unsuccessfully to the wild each year without record); it also probably underestimates the occurrence of ecological effects, which is based on subjective evidence. Of 3141 introduction records in the DIAS database, it is "unknown" whether the species succeeded in establishing in 13.9% of the records and whether it had ecological effects in 80%. The number of species imported into a country is also largely unknown and not reported in DIAS. Finally, Williamson (1996) described the tens rule for a "pest" transition (i.e., established introduced species causing negative socioeconomic effects) instead of the invasion transition. For all these reasons, this paper will focus on the establishment transition and will only briefly discuss the introduction transition and the existence of ecological effects. In the Discussion, we comment on the potential for bias in the overall percentages, which should be regarded with caution. Our approach is comparative, rather than to estimate absolute figures, because we consider that when comparing continents, countries, or the different roles played by a country's area, latitude, or population density, the general conclusions are unaffected by the biases.

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Table 1. Invasion transitions of the 10 most frequently introduced aquatic species in the world plus 2 additional species (obtained from the Food and Agriculture Organization's Database on Introductions of Aquatic Species).

	Number of introductions	Percentage established	*P*	Percentage causing ecological effects	*P*
Oreochromis mossambicus	172	85.9	<0.0005	81	0.26
Cyprinus carpio	124	82.0	0.91	86	0.47
Oncorhynchus mykiss	99	53.8	0.38	88	0.51
Ctenopharyngodon idella	91	11.3	0.06	60	0.71
Hypophthalmichthys molitrix	79	26.8	0.20	75	0.24
Oreochromis niloticus	78	70.2	<0.0005	75	0.25
Gambusia spp.	67	96.8	0.43	50	0.15
Micropterus salmoides	64	72.9	0.78	86	0.19
Aristichthys nobilis	55	19.6	0.27	80	0.48
Carassius auratus	54	92.3	0.63	75	0.37
Lepomis gibbosus	25	91.3	0.01	71	—
Procambarus clarkii	24	88.9	0.62	86	0.74

Note: The number of introductions refers to introductions to different countries. Several introductions of the same species into the same country are not considered. The *P* values correspond to *G* tests of independence of establishment or presence of ecological effects (yes/no) in different continents.

Following Williamson (1996), percentages refer to the total number of species in the previous transition, e.g., the percentage of those species which established (not of the total which were introduced) that had ecological effects. Unfortunately, the effect of introduction effort within a country cannot be analyzed because in the DIAS database, only the first introduction is given for each country.

We prepared three data sets. One contained all the world's introductions of the 10 most frequently introduced aquatic species plus 2 aquatic species regarded as very invasive in Europe, the red swamp crayfish (*Procambarus clarkii*), which is the second most frequently introduced crustacean, and the pumpkinseed sunfish (*Lepomis gibbosus*) (Keith and Allardi 1998; García-Berthou and Moreno-Amich 2000*a*) (Table 1). We added to this database the continent of introduction. With this data set we described the invasion rates of the different species and analyzed whether they varied with the continent of introduction and year. The second data set was a transition matrix of the total number of species introduced from one given European country to another. The countries on other continents were not specified, so pool regions (continents) were used instead. A correspondence analysis (CA) of this transition matrix (38 × 38 countries/continents) showed the pathways of introduction within Europe and the main sources of variation. For European countries we added the country's area, latitude, longitude, gross domestic product per capita (GDPC), and population density (from Central Intelligence Agency 2001) to analyze their effects on the number of introductions received and given. The third data set consisted of all introductions (264 introductions of 123 different aquatic species) to six large European countries at contrasting latitudes: United Kingdom (UK), France, Spain, Sweden, Germany, and Italy. The objective was to compare the invasibility of large countries (i.e., to partial out the effect of country size but use countries large enough to avoid low numbers of introductions) at contrasting latitudes (and hence contrasting water availability and environmental features).

Statistical analyses

Contingency tables were analyzed with *G* tests of independence. To test the effect of a quantitative predictor (e.g., year of introduction) on a qualitative response variable (e.g., the percentage successfully established or the percentage causing ecological effects) we used logistic regression; we considered the models both with and without categorical factors (continent). For logistic regression, we report the χ^2 value (and its associated *P* value) that compares the full model with the constant-only model, and we also report Nagelkerke's R^2, which is similar to the ordinary determination coefficient (Tabachnick and Fidell 2001).

Multiple linear regression was used to relate the number of introductions to a country's area, latitude, and longitude. Stepwise selection procedures were used (although similar results were obtained without selection procedures). We used the square of latitude and longitude in the models to test for nonlinear, parabolic responses (i.e., polynomial regression). Latitude was centered (i.e., the mean was subtracted) before squaring it to reduce collinearity. The adjusted coefficient of determination, which corrects the overestimation due to the number of variables, is reported (Tabachnick and Fidell 2001).

Path analysis was used to better include intervening variables that may mediate between number of introductions and geographical characteristics, such as GDPC and population density. Path analysis is strongly related to regression analysis but has the following advantages: automatic computation of indirect effects, the ability to treat all equations in a single model, the ability to deal with non-recursive systems, and the availability of tests of significance of all the potentially omitted parameters. We estimated the recursive saturated model (i.e., assuming a causal order among variables and estimating all possible effects that are coherent with this order) on

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the correlation matrix using the maximum-likelihood estimator for general structural equation models, as available in the program LISREL 8.54 (Du Toit and Du Toit 2001). Then, we went into a specification search process by removing one by one nonsignificant paths (with a t value lower than 2), by assessing the interpretability of the results after each modification, and by testing each time the significance of all parameters omitted in previous steps, both jointly by means of a likelihood ratio test and individually by means of Lagrange's multiplier tests, also known as modification indices. This corresponds to the model-generating strategy of structural equation modelling defined in Jöreskog (1993).

CA was used to ordinate and summarize information consisting of large contingency tables of number of introductions (species × continent and country of origin × country of destination). CA is similar to principal component analysis but performs better with matrices of count data with many zeros that are the norm in community ecology (Jongman et al. 1995). Transformations are generally not necessary in CA, since counts are implicitly weighted by row and column totals (usually species and sample totals). The results of CA are usually presented in separate plots or a joint plot of row and column scores for the first dimensions, which explain most of the variation. Close scores in the CA plot correspond to similar entities, and the relationship between rows and columns of the matrix is also displayed, i.e., closer species scores indicate species disproportionately introduced to a given continent (which will also have scores close to the species scores in the plot). All statistical analyses (except path analysis) were computed with SPSS version 11.5 (SPSS Inc. 2002).

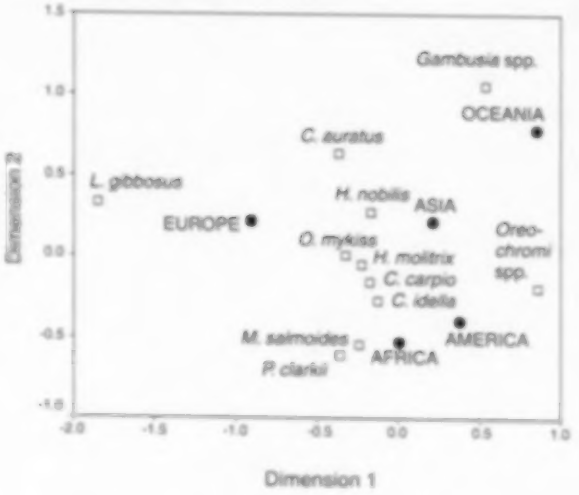
Results

The most frequently introduced aquatic species

According to the DIAS database, the 10 most frequently introduced aquatic species in the world are all freshwater fishes, which have been introduced into over 50 countries (Table 1). There are significant differences among continents in the introduction intensity of different species ($G_{[40]} = 148.1$, $P < 0.0005$) and a CA (Fig. 1) shows that the pumpkinseed sunfish has been introduced mostly to Europe, mosquitofish (*Gambusia* spp.) mostly to Oceania, tilapias (*Oreochromis* spp.) mostly to America and Oceania, the largemouth bass (*Micropterus salmoides*) mostly to Africa, and the bighead carp (*Aristichthys nobilis*) mostly to Asia. Of the 25 introductions of pumpkinseed sunfish (Table 1), 72% were to European countries; of the 172 introductions of tilapias, 34% were to America, 16% to Oceania, and only 6% to Europe; of the introductions of mosquitofish, 27% were to Oceania; of the introductions of goldfish (*Carassius auratus*), 37% were to Europe and only 7% to Africa; of the introductions of largemouth bass, 30% were to Africa and only 12% to Asia; and of the introductions of bighead carp, 40% were to Asia and only 9% to Africa.

There is also significant variation in establishment rates among continents (Table 1). Over 65% of introduced tilapias successfully established, except in Europe, where all introductions have failed. The introductions of pumpkinseed sunfish were all successful, except two out of three attempts in

Fig. 1. Correspondence analysis of the number of introductions, by continent, of the most frequently introduced aquatic species (□, species scores; ●, continent scores). The two first dimensions explain 75.5% of the variance.



Africa and Asia. The probability of establishment is lower for the grass carp (*Ctenopharyngodon idella*), bighead carp, and silver carp (*Hypophthalmichthys molitrix*). For the tilapias there is also a saturation effect, and a logistic regression function shows that about 20% of the most recent introductions have failed (mostly in Europe) in contrast to the earliest introductions, which were all successful (Fig. 2).

Over 50% of the successful introductions of the 12 species had ecological effects, and the occurrence of ecological effects does not depend significantly on the destination continent (Table 1) or year of introduction (logistic regression, $P > 0.05$). For these 12 species, there was no significant relationship between the number of introductions and the percentage established ($r = -0.03$, $n = 11$, $P = 0.93$) or the percentage causing ecological effects ($r = -0.09$, $n = 11$, $P = 0.79$).

Invasion rates in six European countries

The number of species introduced is much lower in Sweden than in the other five large countries assessed, although no significant effect of latitude was obtained with multiple regression (but see the next section). The mean percentage established of the 123 aquatic species in the six countries is above 50%, and the average is 63% (Table 2). There is some suggestion that the percentage established varies among countries ($G_{[5]} = 10.1$, $P = 0.072$), establishment success being lowest at higher latitudes and longitudes ($r = -0.82$, $n = 6$, $P = 0.047$). The frequency of ecological effects does not vary significantly among countries ($G_{[5]} = 1.6$, $P = 0.12$) and is 69%, on average. There is no significant relationship between the number of introductions and the percentage established ($r = 0.51$, $n = 6$, $P = 0.30$) or the percentage causing ecological effects ($r = 0.01$, $n = 6$, $P = 0.98$).

Introduction pathways within Europe

The first two dimensions of a CA of the transition matrix of introduction counts (38 × 38 countries) explained 32.4%

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Fig. 2. Historical variation in establishment success of tilapias, (*a*) *Oreochromis mossambicus* and (*b*) *Oreochromis niloticus*, in Europe. The data points are recorded introductions (1 = established; 0 = not established) and the line shows the probability of establishment success predicted by logistic regression.

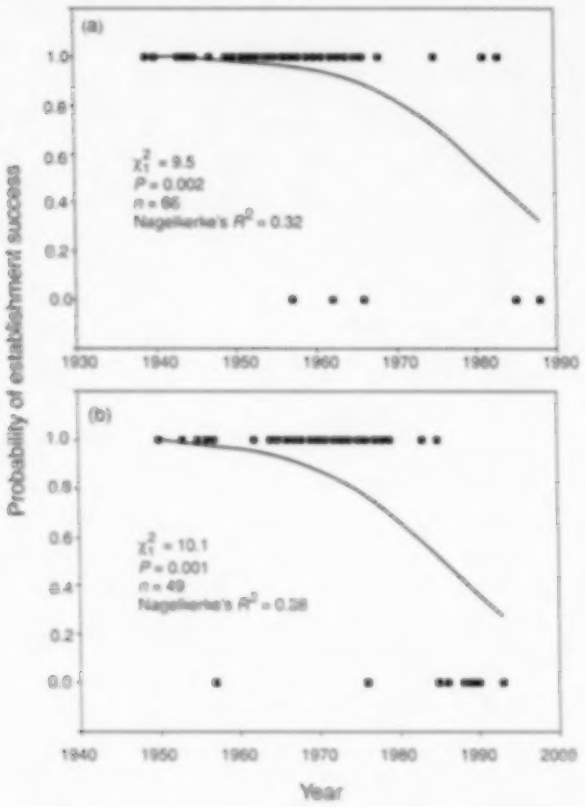
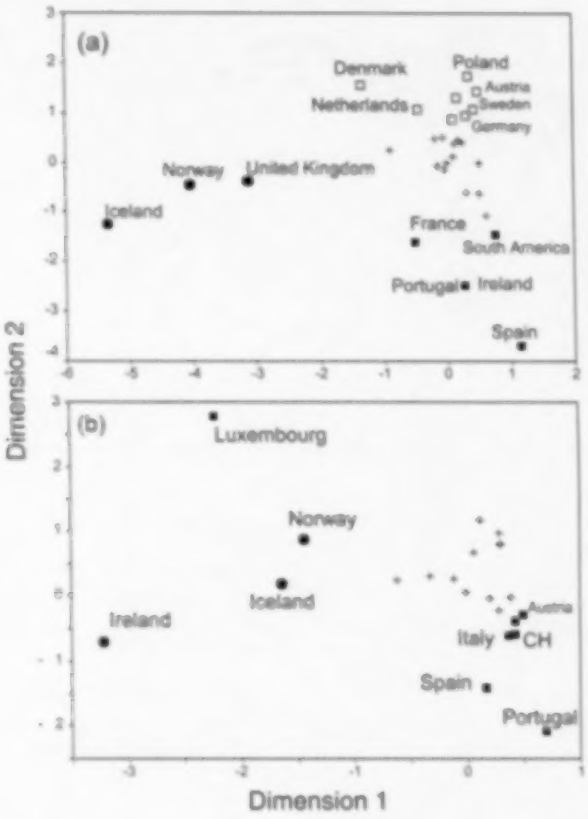


Table 2. Invasion transition rates in six large European countries at contrasting latitudes.

	Number of introductions	Percentage established	Percentage causing ecological effects
United Kingdom	47	62.8	80
France	63	69.5	83
Spain	42	80.0	36
Sweden	16	43.8	67
Germany	40	67.6	100
Italy	56	55.6	71

of the variation (Fig. 3). The CA summarizes introduction-pathway relationships among European countries. Three regions of origin of introductions are distinguished by *k*-means clustering from the rest of the CA origin scores (Fig. 3*a*): (1) northwestern European countries, dominated by the UK. The CA destination scores (Fig. 3*b*) distinguish Ireland as the main destination. The UK gave 14 of the total of 26 introductions to Ireland; (2) northern countries, dominated by Germany, that provide species to many different countries; and (3) southwestern countries, with France as the main donor of species to Spain and Portugal.

Fig. 3. Correspondence analysis of the transition matrix of introductions (number of species introduced from one country to another). Scores for (*a*) origin and (*b*) destination countries are given. Countries with different symbols are in different clusters obtained by *k*-means clustering: only the countries that are most differentiated are labelled; a plus sign (+) denotes countries with central scores (not differentiated by the dimensions). CH, Switzerland.



The CA origin scores (Fig. 3*a*) distinguish large countries such as the UK, Germany, and France that are not distinguished among the CA destination scores (Fig. 3*b*). This reflects the strong asymmetry in the exchange of non-indigenous species (NIS), with strong unidirectional pathways. Of the 26 species introduced from the UK, 14 went to Ireland; in contrast, Ireland provided only one introduction (not to the UK but to Spain). Of the 18 species introduced from France, 10 went to Spain, whereas the 3 introduced from Spain went to Portugal.

There are, therefore, clear introduction pathways in Europe (Fig. 4). The many species of North American origin enter France, the UK, Germany, and Italy independently. There is strong introduction intensity from the UK to Ireland, from France to Spain, and from Spain to Portugal, illustrating "stepping stone" invasion patterns. Germany acts

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Fig. 4. The main introduction pathways of aquatic species within Europe. The fractions denote the number of species introduced from one country to another (numerator) followed by the total number of introductions received, excluding those of unknown origin (denominator). For instance, of the 22 species of known origin introduced to Ireland, 14 (i.e., 64%) came from the United Kingdom. Relationships involving 10 or more introductions are indicated by thick arrows. Less important relationships have been omitted.

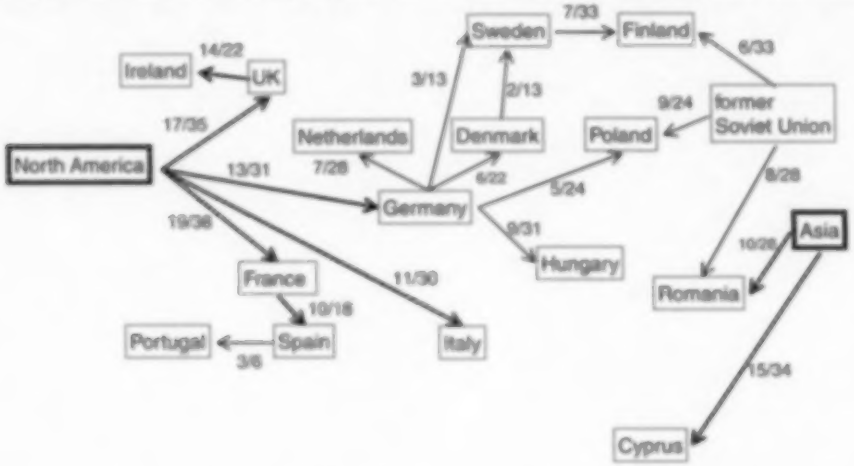

Table 3. Multiple linear regression models (stepwise selection procedure) of the number of introductions with country area and latitude.

Model	R^2_{adj}	P
log(GIVEN + 1) = –10.4 + 0.40 log AREA – 0.38 LAT – 0.004 LAT^2	0.31	0.017
log RECEIVED = –0.376 + 0.44 log AREA – 0.0002 LAT^2	0.48	0.001
log RECEIVED = –0.17 + 0.35 log AREA + 0.23 log(GIVEN +1) – 0.0002 LAT^2	0.56	<0.0005

Note: GIVEN is the number of species introduced from the country to other countries; RECEIVED is the number of species introduced to the country; AREA is the area of the country (km^2); LAT is latitude (deg.); log is the decimal logarithm; and R^2_{adj} is the adjusted coefficient of determination (n = 23 European countries). All the regression coefficients were significant ($P < 0.05$).

as a source of NIS for many countries in central and eastern Europe. Besides North America, most NIS originate from Asia or the former Soviet Union and enter through Romania and Poland.

Effects of a country's area and latitude on the number of introductions

Multiple linear regression analysis shows that the number of introductions originating from a given country is significantly and independently related both to its area and its latitude (Table 3). The standardized regression coefficients and their P values show that the parabolic latitudinal effects are more important than the country's area. Most species are introduced from midlatitude Europe and secondarily from large countries, whereas few species are introduced from either northern or southern latitudes.

The number of introductions received by a country is also related to its area and latitude (Table 3). However, area is more important here than latitude, and the latitudinal effect is monotonic, i.e., southern and central countries receive similar numbers of introductions and only Nordic countries receive fewer.

The relationship between the number of introductions received and given by a country is significant ($P < 0.05$; Fig. 5) and the slope is significantly different from a 1:1 relationship ($P < 0.05$) because most countries receive many more species than they give, except for five large, rather midlatitude countries. There is a positive effect of a country's area on the ratio of received to given introductions and a quadratic effect of latitude (Table 3), and beta coefficients show that area is the most important factor in this relationship and the number of introductions given is the least important. The ratio of received to given introductions is higher in southern countries and lower in northern countries, and three types of country can be summarized (Fig. 5): "recipient and donor" countries: these give and receive many introductions, and are mainly large, rather midlatitude countries such as France, Germany, the UK, and Hungary; "recipient" countries: these give few species but receive many, and include most of the countries, although southern countries receive proportionally more species; and "neither recipient nor donor" countries (only Iceland and Luxembourg). No country is only a "donor".

Effects of population density and GDPC

We obtained a relatively low degree of collinearity among the variables (Table 4), in part through centering latitude before squaring it, using GDPC instead of total gross domestic product, and using population per square kilometre instead of total population. All the effects shown (with arrows) in the model finally obtained (Fig. 6) were significant ($P < 0.05$). The model did not significantly deviate from the ini-

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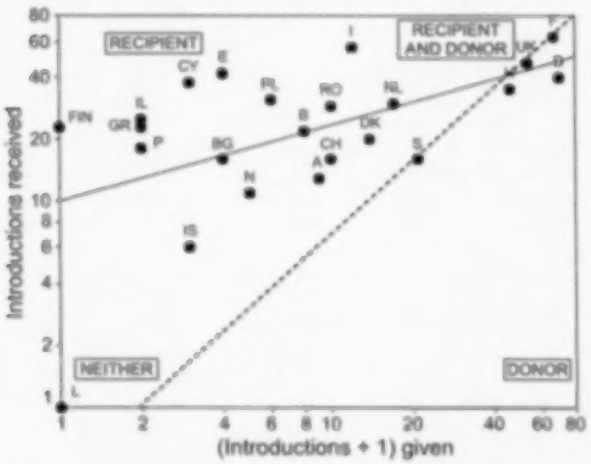
Table 4. Correlation matrix of the variables for the 23 European countries analyzed.

	log RECEIVED[a]	log GIVEN[b]	GDPC[c]	Population density[d]	log AREA[e]	Latitude (deg.)	Latitude squared[f]
log RECEIVED[a]	—	0.008	0.031	0.407	0.003	0.162	0.601
log GIVEN[b]	0.539	—	0.801	0.065	0.071	0.838	0.026
GDPC[c]	–0.449	–0.056	—	0.326	0.055	0.108	0.785
Population density[d]	0.182	0.391	0.215	—	0.179	0.538	0.004
log AREA[e]	0.584	0.384	–0.405	–0.291	—	0.372	0.666
Latitude	–0.302	0.045	0.344	–0.135	0.195	—	0.300
Latitude squared[f]	–0.115	–0.462	0.060	–0.581	0.095	0.226	—

Note: Values below the diagonal are correlation coefficients; values above the diagonal are *P* values.
[a]log RECEIVED is the number of species introduced to the country ($\log_{10}$ transformed).
[b]log GIVEN is the number of species introduced from the country to other countries ($\log_{10}$ (x +1) transformed).
[c]Gross domestic product per capita (US$).
[d]Number of inhabitants·km^{-2}.
[e]Area of the country (km^2; $\log_{10}$ transformed).
[f]Previously centered.

Fig. 5. Relationship of the number of introductions received with the number of introductions given by European countries (A, Austria; B, Belgium; BG, Bulgary; CH, Switzerland; CY, Cyprus; D, Germany; DK, Denmark; E, Spain; F, France; FIN, Finland; GR, Greece; H, Hungary; I, Italy; IL, Ireland; IS, Iceland; L, Luxembourg; N, Norway; NL, the Netherlands; P, Portugal; PL, Poland; RO, Romania; S, Sweden; UK, the United Kingdom). The linear regression function (solid line) and the 1:1 relationship (broken line) are also shown.



tial saturated model (likelihood ratio test = 11.9, df = 10, $P = 0.29$). The maximum Lagrange multiplier test for individual omitted parameters was also nonsignificant ($\chi^2_1 = 1.9$, $P = 0.17$) R^2 values were 0.350 for GDPC, 0.337 for population density, 0.423 for introductions given, and 0.632 for introductions received. The following points emerge from the results of path analysis (Fig. 6): (*i*) the country's area (the most important explanatory variable) and population density affect the numbers of species both received and given. No effect of GDPC was found; (*ii*) there is a direct, negative effect of latitude on the number of introductions received. In contrast, the previously observed quadratic effect of latitude on the number of introductions given is mediated by population density (countries in central Europe are the most densely populated and give most of the introductions); (*iii*) when controlling for the explanatory variables, no significant relationship between the number of species given (GIVEN) and received (RECEIVED) is left, therefore the number of introductions in a country per se does not affect its exportation rate, despite the apparent correlation. No significant correlation is found between the error terms of GIVEN and RECEIVED, hence supporting their randomness and the completeness of the model.

Discussion

Introduction pathways

There are significant differences among continents in the frequency of introductions of different species. For instance, the pumpkinseed sunfish has been introduced mostly to Europe, whereas mosquitofish are widespread in Oceania. There are also some significant intercontinental differences in establishment success but only for a few species, and establishment success is generally high and less important than the introduction effort (see the previous section). Therefore, the differential introduction frequency is mediated by historical and sociological factors rather than ecological factors. For instance, the pumpkinseed sunfish would be expected to adapt well to many African countries (there were only two introduction records in DIAS), where the largemouth bass, a species often cohabiting with the pumpkinseed sunfish in its native range, is widespread. Similarly, mosquitofish are relatively less introduced in Africa and South America but may establish successfully in many countries.

MacIsaac et al. (2001) have recently identified several indirect corridors within Europe that facilitate the invasion of the Laurentian Great Lakes by species mostly from the Ponto-Caspian region. These corridors go from the Black, Azov, and Caspian seas to the North and Baltic seas through the Danube, Dnieper, and Volga rivers (in a northwesterly direction) (Bij de Vaate et al. 2002). These corridors are not observed in our results because they referred to invertebrates (amphipods and crustaceans) with some degree of salinity tolerance and accidentally introduced through ballast water (Ricciardi and Rasmussen 1998; Ricciardi and MacIsaac 2000; MacIsaac et al. 2001), whereas most of the introduced aquatic species in Europe are fishes of freshwater habitats

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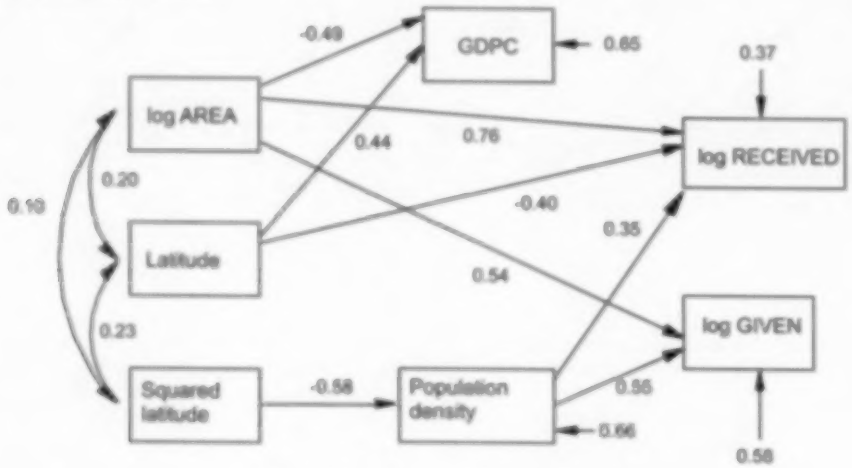
Fig. 6. Path diagram of the final model. Standardized estimates of significant direct effects (single-headed arrows originating from a given variable) and standardized error variances ($1 - R^2$; arrows not originating from variables) are shown. Variables are as follows: log RECEIVED is the number of species introduced to the country ($\log_{10}$ transformed); log GIVEN is the number of species introduced from the country to other countries ($\log_{10}(x + 1)$ transformed); GDPC is the gross domestic product per capita (US$); population density is the number of inhabitants·km^{-2}; log AREA is the area of the country (km^2; $\log_{10}$ transformed); latitude is in degrees; the square of the latitude is previously centered.



and were intentionally introduced. This suggests the existence of two opposing pathways in Europe for intentional (freshwater fishes) and accidental introduction (euryhaline invertebrate species). In contrast to other taxa such as plants and terrestrial vertebrates, freshwater species introduced to Europe come mostly from North America (Welcomme 1991; Vilà et al. 2001) and enter through midlatitude countries in western Europe (France, the UK, and Germany) and from there go mostly to southern Europe. In contrast, the numerous Eurasian euryhaline species (mostly invertebrates but also a few fish species) that have invaded North American waters come from the Ponto-Caspian region through northern Europe, invading Europe in a northwesterly direction.

The species richness – area relationship is one the main topics of ecology and a pervasive pattern at all scales. It is also observed with the number of aquatic NIS introduced in Europe. Interestingly, the numbers of introductions received (RECEIVED) and given (GIVEN) by a country are mediated by different mechanisms: RECEIVED depends mostly on country area and secondarily on latitude, whereas GIVEN depends on population density and area. For RECEIVED, area is a synthetic measure of the diversity of habitats and ecosystems available and the number of river basins (where independent introductions are carried out for freshwater fishes). In contrast, the country of origin (GIVEN) depends mostly on population density and this factor explains the quadratic effect of latitude. For worldwide (received) introductions of terrestrial plants, Dalmazzone (2000) also found that population density was more important than GDPC, but she found some significant effects of GDPC, which is more logical for species accidentally introduced by passive transport, such as most terrestrial plants. The differential influences of GDPC versus population density on passively versus actively transported invasive species should be tested further; they might work as indicators of propagule pressure.

Invasion transition rates

Our results show that the establishment rates of aquatic species in Europe, at least for freshwater fishes, deviate from Williamson's tens rule. The percentage established of the 123 aquatic species in six contrasting European countries is, on average, 63% (167 of 264). This figure might be biased, owing to unrecorded unsuccessful introductions. However, the bias seems unlikely to be great enough to fit within the 5%–20% range that would be expected according to Williamson's tens rule, at least for freshwater fishes. We would need some additional 571–3076 unrecorded, unsuccessfully introduced species, or even more, because there are also many established NIS that are not recorded in DIAS. Williamson and coauthors (Williamson 1996; Williamson and Fitter 1996) report several cases that justify the tens rule and some examples that deviate from it, but not freshwater fishes. Our results confirm that intentional introductions (such as previously shown with edible crop plants and biological control) would be expected to have establishment success over 20%, on average.

Most of the 10 most frequently introduced species in the world have establishment success (70%–97%) above the average, but a few, such as the grass carp (11%), bighead carp (20%), and silver carp (27%), display much lower success because of strict breeding requirements that usually impede their naturalization (Lever 1996). These establishment rates below the average of aquatic species in six European countries (63%), combined with the high introduction rates of these three species, confirm the importance of propagule pressure, particularly in intentionally introduced species (Mack 1996; Williamson 1996; Kolar and Lodge 2001). Additionally, the correlations between number of introductions and establishment success were very low and clearly nonsignificant both among species and among countries. Therefore, the two invasion transitions are quite independent (see also Kolar and Lodge 2001, 2002) and the introduction transition is more decisive than the estab-

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lishment one, i.e., widely established species become so not because of a better establishment capability but because their introduction was attempted in many countries.

We did not evaluate the first 10 of the tens rule, i.e., that about 10% of the imported species are introduced to the wild, because no data were available. Too few data are available on this first transition (Williamson 1996; Williamson and Fitter 1996) to call it a rule, but it seems likely that aquatic species in Europe, or at least freshwater fishes, also deviate from 10% but in the opposite direction. The number of aquatic species recorded as having been introduced (successfully or not) in the wild in six large European countries was 123 in total (with a maximum of 65 in a single country), and the number of imported species should be between 615 and 2460 (or below 1300 for a single country) to fit the first of the tens rules. In contrast, it has been estimated that many thousand species of freshwater, brackish water, and marine protists, plants, and animals are transported at any moment in the ballast water of ships (Carlton and Geller 1993). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates the world traffic of more than 30 000 species of animals and plants that are threatened or nearly so. There are about 25 000 fish species and many more aquatic invertebrates; popular atlases of aquarium fish (likely to be regularly imported to Europe) contain over 800 species. Therefore, many thousands of aquatic species are likely imported to Europe every year and a very small selection of them are introduced (intentionally or not) to the wild.

The occurrence of ecological effects caused by NIS was 69%, on average. All the most frequently introduced species were more likely to produce ecological effects except the grass carp and the mosquitofish. The lowest percentage for mosquitofish is unconvincing because their potential ecological impact is well known (Meffe and Snelson 1989; Arthington 1991); this pattern might be due to their smaller size and the difficulty of detecting their predation on small fish, eggs, and other organisms (Nesbit and Meffe 1993; García-Berthou 1999) or to bias in the DIAS database with this transition (see Methods). Although strong ecological impacts are characteristic of introduced freshwater fishes (Williamson 1996), it has not been reported that they display such high percentages of establishment and ecological effects. In a review by the US Congress Office of Technology Assessment (1993), the percentage of harmful NIS in the USA ranged between 4% and 19% and was lowest for fishes (4/111) and highest for terrestrial vertebrates and molluscs, but this corresponds to Williamson's pest transition.

Management implications

We have identified clear introduction pathways of aquatic species into Europe that have profound management implications. Introductions of new species to European countries start in large, central Europe countries; from there they proceed to southern and, secondarily, northern latitudes. Further introduction pathways were identified that highlight where prevention efforts should be concentrated, e.g., most species introduced to Spain come from southern France (see also García-Berthou and Moreno-Amich 2000*b*), most species introduced to Portugal come from western Spain, etc. Species already introduced to the usual country donor should be of most concern (MacIsaac et al. 2001) because they will be the "next" introduction and prevention plans should be in place (e.g., their transport should be effectively banned) and the first introductions should be eradicated, if this is feasible, before the invasion becomes irreversible. Most of these introductions are illegal nowadays and the pathways show the entry routes where more control should be implemented.

Introduction prevention should be regulated at all organizational levels; because the establishment transition is less crucial than the introduction one, it is essential to avoid the introduction of new species to new continents. Because illegal or accidental introductions to new countries within a continent (or new basins within a country) are difficult to avoid, legislation and implementation should be internationally coordinated. Previous success and, to a lesser extent, taxonomy are relatively good predictors of invasive potential (Mack 1996; Williamson 1999), and species and families with previous invasive histories should be of special concern. For terrestrial plants, it has been shown that it is probably more efficient to control new, nascent foci than the main area invaded (Moody and Mack 1998).

Regression analysis allows the identification of three types of country: recipient and donor, recipient, and neither recipient nor donor. The recipient and donor type comprises four countries where the exchange of new NIS is more intense and should be more controlled. From a conservation point of view, it is of concern that southern countries, which within Europe contain a higher proportion of endemic fishes (Almaça 1995; Bianco 1995; Elvira 1995), are among the countries that receive more NIS (recipients). Given the lower availability of water in southern Europe and the higher number of endemics, a stronger ecological impact of NIS seems likely. In contrast, northern Europe has a lower number of native and endemic species and also a lower number of NIS. This pattern is similar to that in the United States, where states with fewer native species considered desirable gamefish (the western states and New England) have received more NIS (Rahel 2000). The control and survey of fish NIS is an urgent need, particularly in southern Europe. We know very little about the ecological impact of most introduced species (Blossey 1999; Parker et al. 1999; Ruiz et al. 1999), including freshwater fishes (Keith and Allardi 1998; García-Berthou and Moreno-Amich 2000*b*; Crivelli 2002), and more funds, attention, and long-term monitoring should be devoted to this important environmental problem.

Acknowledgments

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In situ measurement of tissue turnover and energy conversion efficiencies in lake trout (*Salvelinus namaycush*) using a novel toxicokinetic approach

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Abstract: We determined polychlorinated biphenyl (PCB) elimination patterns in lake trout (*Salvelinus namaycush*) from Lake Ontario using biomonitoring data collected from 1977 to 1993. The in situ elimination rates of these persistent pollutants were found to describe tissue turnover rates in lake trout. A model relating tissue turnover rates and endogenous energy conversion efficiencies revealed that chemical elimination in larger organisms is primarily regulated by food limitation and bioenergetic mechanisms rather than chemical kinetics. Lake trout approximately 2500 g and larger were observed to have higher PCB elimination rates than smaller fish as a result of increased lipid mobilization to supplement metabolic demands due to increased time spent foraging. This study concludes that the growth and production of large predators in Lake Ontario are regulated by the bioenergetic constraints of searching for prey in a food-limited environment. We also demonstrate that persistent organic pollutant kinetics can describe the proportion of endogenous energy required to support metabolism and production, thus providing important in situ measurements of bioenergetic processes.

Résumé : Des données de surveillance biologique de 1977 à 1993 nous ont servi à déterminer les patrons d'élimination des biphényls polychlorés (BPC) chez le touladi (*Salvelinus namaycush*) au lac Ontario. Les taux d'élimination sur place des ces polluants persistants représentent les taux de remplacement tissulaire chez le touladi. Un modèle qui met en relation les taux de remplacement tissulaire et les efficacités endogènes de conversion énergétique montre que l'élimination chimique chez les organismes de plus grande taille est principalement contrôlée par la limitation de nourriture et les mécanismes bioénergétiques que par la cinétique chimique. Les touladis d'environ 2500 g et de taille supérieure ont des taux d'élimination de BPC plus élevés que les poissons plus petits à cause d'une mobilisation plus forte des lipides pour assurer les besoins énergétiques requis par un temps plus long passé à la recherche de nourriture. Notre étude conclut que la croissance et la production des grands prédateurs dans le lac Ontario sont contrôlées par les contraintes bioénergétiques reliées à la recherche de proies dans un milieu où la nourriture est limitée. Nous démontrons aussi que la cinétique des polluants organiques persistants peut décrire la proportion de l'énergie endogène qui est requise pour supporter le métabolisme et la production, ce qui fournit des mesures in situ des processus bioénergétiques.

[Traduit par la Rédaction]

Introduction

Lake trout (*Salvelinus namaycush*) have been used as a pollutant biomonitor throughout the Great Lakes watershed since the late 1970s. Concentrations of persistent chemicals, such as polychlorinated biphenyls (PCBs), in this species have declined substantially since pollutant abatement programs began (Huestis et al. 1996; Devault et al. 1996). Fluctuations in Lake Ontario lake trout PCB levels during these monitoring programs have been attributed to substantial changes in population dynamics of the alewife forage base (Borgmann and Whittle 1991). Such observations suggest

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Table 1. Summarized biological and ΣPCB concentration data for Lake Ontario lake trout (*Salvelinus namaycush*).

Size class (g)	*N*	Lengths (average) (cm)	Masses (average) (g)	No. of immature fish	Age range (years)	Proportion males/females	Average ΣPCB (ng·g wet mass^{-1})	Average k_2 (year^{-1})[a]	Average $t_{1/2}$ (years)[b]
<1500	24	35–53 (47)	457–1456 (1125)	2	3–5	13/9	3684.6	0.04	33
1500–2500	60	49–65 (57)	1519–2485 (2033)	0	4–5	45/15	3054.4	0.06	19
>2500	25	56.5–67.5 (63)	2574–3825 (2999)	0	4–5	10/15	4561.9	0.08	12

[a]PCB elimination rate constant.
[b]PCB congener half-life.

that energy flow in aquatic food webs can be tracked by understanding the processes that regulate pollutant burdens in top predators.

Bioenergetics models have been developed and evaluated in attempts to understand implications of changes in prey densities and pollutant burdens on chemical accumulation in species including chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) and lake trout (Jackson and Schindler 1996; Madenjian et al. 1998*a*, 1998*b*). These studies have primarily focused on pollutant food web transfer efficiencies rather than the importance of bioenergetic processes in regulating observed spatial and temporal trends. Empirical relationships dictate that predator–prey interactions, population dynamics, and bioenergetics play important roles in regulating fish pollutant concentrations (Rasmussen et al. 1990). In this context, persistent pollutant data and trends generated from biomonitoring programs have the potential to provide significant insight into the framework of bioenergetics modeling.

The primary route of pollutant exposure and accumulation in large aquatic predators is via the diet (Russell et al. 1999). The lake trout along with brown (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) and chinook and coho salmon are managed populations in Lake Ontario with fisheries management programs maintaining population numbers to support extensive recreational fisheries (Brandt 1986; Great Lakes Fisheries Commission 1994). The primary prey for these salmonids is the alewife (*Alosa pseudoharengus*), and substantial competition exists among these predators for this forage (Brandt 1986). Owing to significant changes in growth, abundance, and general decreases in size and condition of alewives (Rand et al. 1994; O'Gorman et al. 1997), salmonid stocking rates have been reduced to maintain a balance between recreational fishing demands and preservation of alewife stocks (Great Lakes Fisheries Commission 1994). Such management of salmonid stocking rates has also been proposed as a mechanism for reducing the accumulation of persistent pollutants such as PCBs in fish tissues (Stow 1995; Jackson 1996, 1997). Thus, alewives must be considered as both the primary route of chemical exposure and energetic reserve for salmonid predators in Lake Ontario.

Under conditions of high prey densities, surplus consumed energy is stored as lipids and the reliance on these reserves to meet metabolic requirements is low (Hawkins 1985; Jayaram and Beamish 1992). Consequently, specific growth rates are high with rapid assimilation of externally acquired energy into growth (Elliot 1976). As food availability is reduced, the energetic costs of acquiring prey increases owing to extended time and energy spent searching for prey. In this situation, somatic lipid reserves are first mobilized to supplement the metabolic demands of foraging in a prey limited environment (Elliot 1976; Schmidt-Nielsen 1990; He and Stewart 1998). This mobilization or turnover of somatic reserves is related to growth rates and is reflected by seasonal dynamics in somatic energy density (Houlihan et al. 1988). Additionally, changes in metabolic rates and reproductive demands can also alter specific rates of such somatic turnover. Tissue turnover can therefore supplement energetic requirements when exogenous energy does not meet metabolic demand or contribute to production when consumed energy satiates metabolic requirements (He and Stewart 1998).

Lake trout PCB concentrations have declined to a lesser extent than that demonstrated for sediment levels in Lake Ontario (Marvin et al. 2003). These different trends suggest that chemical elimination in biota is tightly coupled with species and population bioenergetics rather than pollutant loadings. The objective of this research was to evaluate the potential to use toxicokinetics models as a measure of bioenergetic parameters in the Lake Ontario lake trout population. The inherent stability of pollutants such as PCBs indicates that these compounds will perform as biologically neutral in situ markers of species and potentially population bioenergetics. More importantly, by using data collected from wild-caught individuals, the results will reflect the energetic consequences of a predator tracking and capturing prey in its native habitat. Ultimately, this kinetic model will be extremely valuable for deciphering species bioenergetics where active long-term pollutant monitoring programs exist.

Materials and methods

The Contaminants Surveillance Program conducted by the Department of Fisheries and Oceans Great Lakes Laboratory for Fisheries and Aquatic Sciences has collected biological data and measured pollutant concentrations in a variety of Great Lakes biota since 1977 (Kiriluk et al. 1997). The chemical and biological data used for this study were from lake trout specimens collected by this program between 1977 and 1993 from the eastern basin of Lake Ontario north of Main Duck Island. Full details of sample collection, preparation, and chemical analysis procedures have been previously summarized (Huestis et al. 1995, 1996). Briefly, individuals were measured for length and mass and sexed with ages estimated from calcified structures removed from each fish. Carcasses were then homogenized whole using a commercial meat grinder and PCB analysis was completed using 5 g of the homogenate. With the exception of 1979 and 1985, concentration data were available for all years

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during the sampling period. Fish were categorized into three size classes (Table 1) selected to cover approximately 1-kg ranges in lake trout mass with biological and chemical data available for 109 individuals consisting of 68 males, 39 females, and 2 immature specimens. Biological and chemical concentration data for each size class were then evaluated to assess trends in lake trout condition and calculate in situ elimination rate constants and half-lives for 38 PCB congeners in their tissues between 1977 and 1993.

PCB congener kinetics calculations

Because of a significant linear relationship ($r = 0.532$, $p < 0.001$) between lake trout body mass and lipid content, PCB congener concentrations were lipid corrected for all lake trout. Elimination rate constants (k_2) for individual PCB congeners were derived from the slopes of the least squares regression of natural log transformed congener concentration values plotted across the 1977–1993 sampling period. The calculations can be expressed as follows:

$$\ln[\mathrm{PCB}] = a(\mathrm{year}) + b \quad (1)$$

$$k_2 = |a| \quad (2)$$

where [PCB] is the lipid corrected PCB congener concentration with a and b representing the regression slope coefficient and constants, respectively. The PCB congener elimination rate constant k_2 is reported in units of per year. PCB congener half-lives ($t_{1/2}$), measured in years, were calculated per eq. 3 following first-order rate kinetics:

$$t_{1/2} = \ln 2/k_2 \quad (3)$$

Bioenergetics modeling calculations

The relationship between lake trout tissue turnover (τ) (joules per joule per year) and body mass followed that calculated by He and Stewart (1998) in eq. 4:

$$\tau = 6.85W^{-0.0407} \quad (4)$$

with W representing lake trout body mass (grams). Energy net conversion efficiencies (NCE) were calculated as a function of both lake trout mass, converted to total body energy, and tissue turnover to growth ratios as described by He and Stewart (1998) in eqs. 5 and 6, respectively:

$$\mathrm{NCE} = 1 - 0.04W^{-0.1723} \quad (5)$$

$$\mathrm{NCE} = 1.30(\tau/G)^{-0.5585} \quad (6)$$

Specific growth rates used in eq. 6 for calculation of NCE were those determined for age- and mass-classes of Lake Ontario lake trout reported by Pazzia et al. (2002).

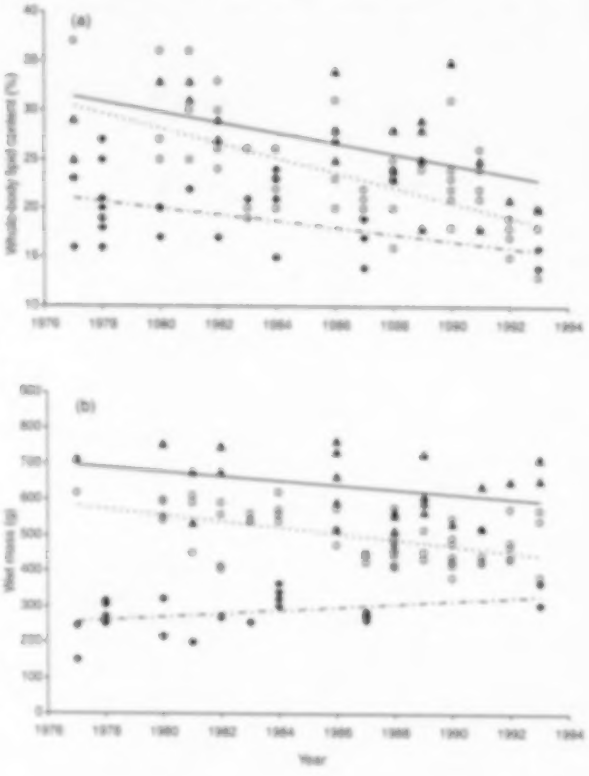
Statistics

All statistics were completed using SYSTAT version 8.0 for Windows (Wilkinson 1998). The criterion for significance in all analyses was $p < 0.05$.

Results

Lipid content in all three size classes of lake trout was observed to decline between 1977 and 1993 (Fig. 1*a*). The most rapid change in lipid content (0.7%·year^{-1} (±0.1%)) was observed for the intermediate (1500–2500 g) size class with losses of lipid content of 0.5%·year^{-1} (±0.2%) and 0.3%·year^{-1} (±0.1%) calculated for the largest and smallest size classes, respectively. Decreases in lake trout body mass (grams wet mass), however, were observed only in the two larger size classes over the study period, while the smallest size class demonstrated an increase in body mass of approximately 18 g·year^{-1} (Fig. 1*b*).

Fig. 1. Trends in (*a*) whole-body lipid content and (*b*) wet mass for three size classes (<1500, 1500–2500, and >2500 g) of Lake Ontario lake trout (*Salvelinus namaycush*) from 1977 to 1993. For both panels, individual lake trout lipid content and wet mass for the respective size classes are represented by ◆, ○, and ▲ with, in increasing order of size, the dot-dashed, dotted, and solid lines representing the least squares regression lines.



For all size classes, PCB congener k_2 values were negatively correlated with increasing chemical hydrophobicity (log k_{ow}) (Fig 2*a*). Highest average PCB k_2 values were observed for the largest lake trout size class, with the smallest individuals having, on average, the lowest k_2 values for the 38 PCB congeners (Table 1). With the exception of PCB 193, significant differences between all PCB k_2 values were demonstrated among the three size classes (analysis of covariance, $p < 0.001$). One-way analysis of variance indicated PCB $t_{1/2}$ values for the <1500 g lake trout size class were significantly longer than those calculated for the largest individuals ($p = 0.034$). No significant differences in PCB

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Fig. 2. Relationships between PCB congener (*a*) elimination rate constants (k_2) and (*b*) half-lives ($t_{1/2}$) and congener octanol–water partition coefficients (log K_{ow}) among three size classes (<1500, 1500–2500, and >2500 g) of Lake Ontario lake trout (*Salvelinus namaycush*). Individual PCB congener elimination rates and half-lives for the respective size classes are represented by ◆, ○, and ▲ with, in increasing order of size, the dot-dashed, dashed, and solid lines representing the least squares regression lines.

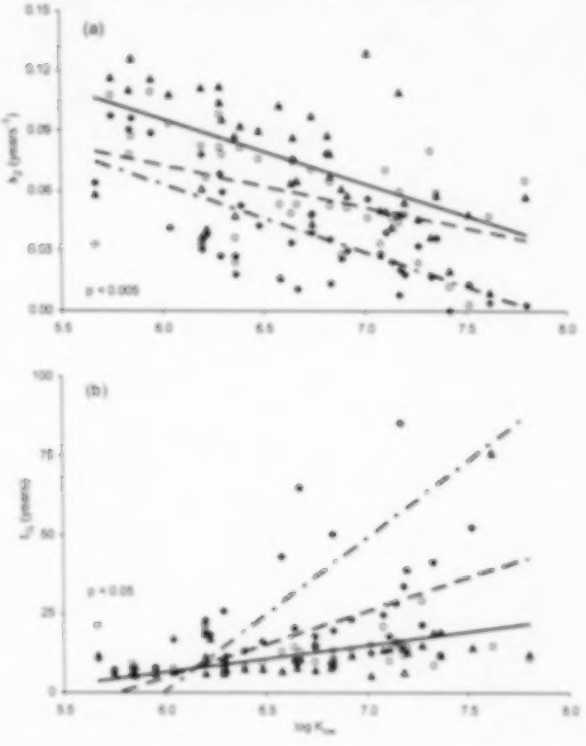
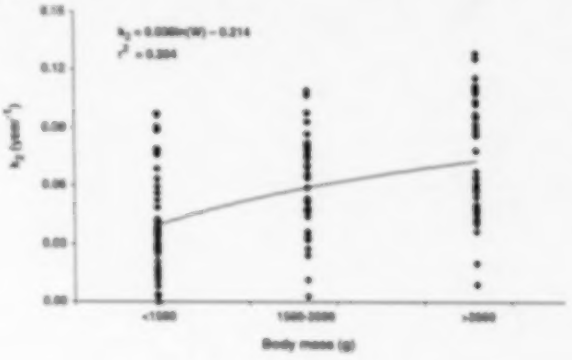


Fig. 3. Relationship between PCB elimination rates (k_2) and Lake Ontario lake trout (*Salvelinus namaycush*) body mass. The solid line represents the best-fit log-linear regression curve for the data.



congener $t_{1/2}$ values were observed between the <1500- and 1500- to 2500-g size classes ($p = 0.684$) or the 1500- to 2500- and >2500-g size classes ($p = 0.209$). Significant differences (analysis of covariance, $p < 0.005$) were also observed between the relationships of PCB k_2 values with their respective log K_{ow} values for the lake trout size classes (Fig. 2*a*).

The highest PCB $t_{1/2}$ values were calculated for the smallest size class with decreases in $t_{1/2}$ values reported for increasing lake trout size class (Fig. 2*b*). Although PCB congener $t_{1/2}$ values were significantly different between the three lake trout size classes, this significance was primarily generated by the difference in $t_{1/2}$ values between the smallest and largest size classes only (Tukey's test, $p = 0.034$). Regardless of size class, however, PCB $t_{1/2}$ values increased with increasing congener log K_{ow}.

Best fit log-linear regression of PCB k_2 values plotted against the size range of lake trout in this study describes a logarithmic curve increasing with body mass (Fig. 3). Most importantly, this function describes the same relationship modeled for tissue turnover rates in mature lake trout over the size range of individuals (Fig. 4*a*). Additionally, the ratio of tissue turnover τ to PCB k_2 values (τk_2) describes the relationship outlined by He and Stewart (1998) in eq. 5 for energy NCE with tissue turnover to growth ratio described for lake trout (Fig. 4*b*). These relationships indicate that bioenergetic processes such as tissue turnover and energy conversion efficiencies regulate chemical elimination in large aquatic predators.

A combined plot of the NCE and tissue turnover curves described by He and Stewart (1998) as a function of lake trout body mass is provided in Fig. 5*a*. Plotting the ratio for lake trout tissue turnover to PCB k_2 values (τk_2) against lake trout body mass with the log-linear curve for k_2 with mass (Fig. 5*b*) yielded a strikingly similar relationship to that found between the NCE and tissue turnover functions (Figs. 5*a* and 5*b*). The intersection points for these curves (described in Figs. 5*a* and 5*b*) differ slightly, however. Lake trout mass at the intersection of the NCE and tissue turnover functions is ~2100 g (Fig. 5*a*). For the plot of the ratio τk_2 and the log-linear curve for k_2 against lake trout body mass, however, the point of intersection between the two curves is at a body mass of ~1800 g (Fig. 5*b*). The differences between these intersection points reflect the nature of the tissue turnover function as described in eq. 4 from He and Stewart (1998) and the bioenergetic mechanism that regulates chemical elimination in lake trout.

Discussion

Considerable changes in the growth and abundance of the Lake Ontario alewife population (O'Gorman et al. 1997) and a general decrease in alewife size and condition were reported during the sampling period of this study (Rand et al. 1994). This species is the primary forage for larger, mature lake trout and substantial competition exists among salmonid predators for this prey base in Lake Ontario (Brandt 1986). It has also been suggested the Lake Ontario alewife population cannot support high densities of large, long-lived predators under heavy stocking pressure (Brandt 1986; Rand and Stewart 1998*a*, 1998*b*). Therefore, to maintain growth rates and lipid content, larger (>1500 g) lake trout will need to consume additional alewife prey while expending additional

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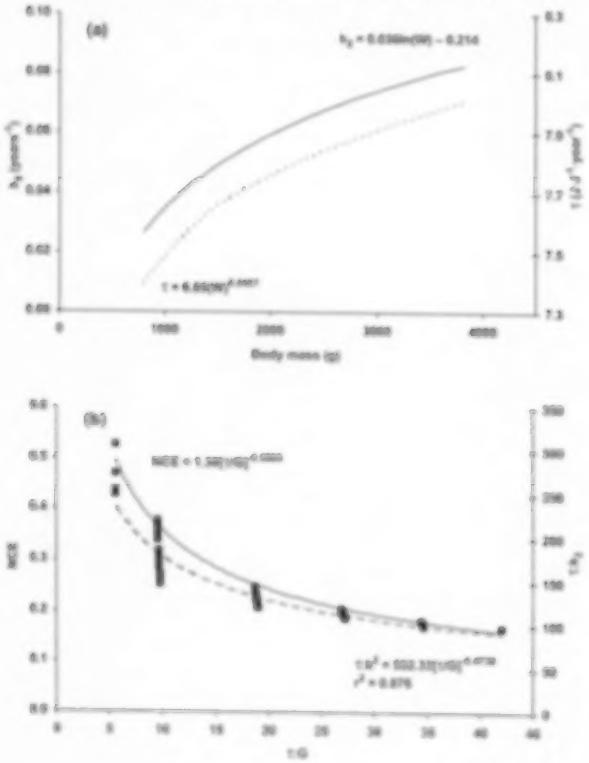
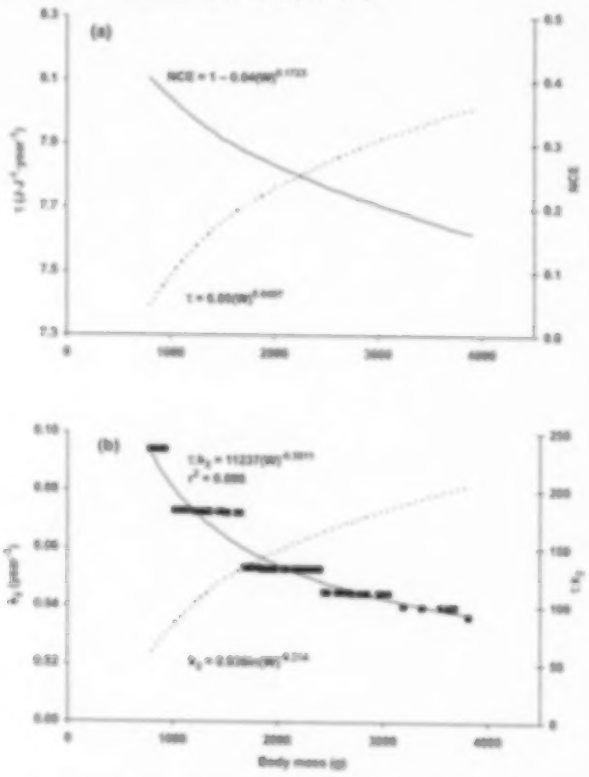
Fig. 4. (*a*) Relationships of PCB elimination (k_2) and tissue turnover (τ) rates with Lake Ontario lake trout (*Salvelinus namaycush*) body mass predicted for mature individuals. Solid and dashed lines describe the functions for PCB elimination and tissue turnover rates, respectively. Allometric relationship for tissue turnover rates follows that previously modeled for mature lake trout by He and Stewart (1998). (*b*) Relationships of energy net conversion efficiency (NCE) and tissue turnover to PCB elimination ratio ($\tau{:}k_2$) with tissue turnover to growth ratio ($\tau{:}G$) in mature lake trout. Solid and dashed lines describe the functions for energy net conversion efficiency and tissue turnover to PCB elimination ratios, respectively. Tissue turnover to PCB elimination rate values for individual fish are represented by ■. Specific growth rates and relationship of energy NCE with tissue turnover to growth ratios for mature lake trout were calculated from existing lake trout bioenergetics models (He and Stewart 1998).



energy foraging (Elrod et al. 1995). The smallest size class of lake trout, however, included individuals considered to be less discriminatory with respect to foraging activities exploiting other prey resources including rainbow smelt (*Osmerus mordax*) and slimy sculpins (*Cottus cognatus*) (Elrod 1983). Combined with the general decrease in size and abundance of larger alewives in Lake Ontario from 1978 to 1990 (O'Gorman et al. 1997), as size-selective predators, the smallest size class of lake trout had a more replete forage base to maintain growth. The increase in body mass observed for the smallest lake trout indicates that these individuals have been able to forage more efficiently than their larger counterparts. The declines in both lipid content and body mass observed for lake trout >1500 g, however, reveal that an energetic bottleneck (Sherwood et al. 2002) in the forage base limits growth and production of these larger individuals in Lake Ontario.

Fig. 5. (*a*) Relationships of net energy conversion efficiency (NCE) and tissue turnover rate (τ) with Lake Ontario lake trout (*Salvelinus namaycush*) body mass (He and Stewart 1998). Solid and dashed lines represent energy NCE and tissue turnover rate functions, respectively. (*b*) Relationships of tissue turnover to PCB elimination ratio ($\tau{:}k_2$) and PCB elimination (k_2) with Lake Ontario lake trout body mass. Tissue turnover to PCB elimination rate values for individual fish are represented by ■. Solid and dashed lines represent tissue turnover to PCB elimination ratio and PCB elimination rate, respectively.



The declines in lipid content reported for all three size classes of lake trout must be considered to reflect not only decreased prey energy densities (Rand et al. 1994) but also the energetic consequences of foraging in Lake Ontario. Endogenous or somatic tissues can be mobilized as an energetic reserve during periods of food limitation to supplement energetic demands and can also contribute to production (He and Stewart 1998). Of the energetic costs required for foraging, searching for prey is considered to be the most expensive (Pazzia et al. 2002). With a reduced proportion of larger alewives in the forage base (O'Gorman et al. 1997), large lake trout will have fewer encounters with preferred prey and spend increased time and energy foraging. Additionally, habitat overlap between salmonid predator and alewife prey is temporally limited owing to confounding effects of thermal stratification requiring predators to search water columns consisting of suboptimal and energetically expensive

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temperature regimes (Roy et al. 2004). To meet these energetic demands, increased reliance on endogenous energy reserves is required, a considerable implication for lake trout existing on a low-energy diet (Elrod et al. 1995). Under these conditions, mobilization of endogenous reserves acts solely to meet such additional energetic costs and will not contribute to endogenous production. The decline in body mass reported between 1977 and 1993 for the two larger size classes confirms this consideration. Combined with the declines in lipid content shown for lake trout >1500 g, these individuals likely spend a disproportionate amount of time searching a depleted forage base at substantial energetic cost.

The declines in both lipid content and body mass were not, however, observed for smaller lake trout (<1500 g). Food limitation is less likely for these individuals, with rainbow smelt and slimy sculpins being potential prey in addition to alewives (Elrod 1983). Having more abundant prey resources increases consumption and decreases the reliance on endogenous reserves (Elliot 1976). The smallest size class maintained body mass over the study period, and this observation confirms the presence of a more robust forage base. However, the decrease in lipid content for the smallest size class likely reflects combined effects. The decreased energy content reported for Lake Ontario prey species (Rand et al. 1994) is partially reflected by the declines in lipid content observed for all size classes of lake trout. In larger fish, a portion of this decline is hypothesized to be due to lipid mobilization as an energy source supplementing the additional metabolic requirements of foraging in a prey-limited environment. For the smallest size class, however, lipid turnover is likely contributing to endogenous production as evidenced by the small increase in lake trout body mass between 1977 and 1993. These declines in lake trout lipid content, however, not only have important consequences regarding conclusions about the state of the Lake Ontario forage base but also result in increased chemical concentration gradients that regulate the PCB elimination patterns observed among these size classes of fish.

Significant differences ($p < 0.005$) were observed among the relationships of PCB k_2 values and their respective log k_{ow} values for the lake trout size classes. This significance was likely due to the steeper slope of the regression line calculated for the intermediate size class (1500–2500 g). The average body mass (2033 g) of this size group is close to the asymptotic value when specific growth and tissue turnover rates are expected to differ substantially from younger, more rapidly growing fish (He and Stewart 1998). These individuals are also expected to approach the mass and energetic density when the onset of reproductive maturity is considered to occur in lake trout populations (Stewart et al. 1983). The subsequent growth, development, and maturation of gonads will increase the specific rate of tissue turnover, but complete recycling of endogenous reserves into somatic or reproductive tissues is limited (He and Stewart 1998). Thus, a loss of the endogenous materials and energy generated by tissue turnover is expected as fish mature (He and Stewart 1998). The difference in the relationship between k_2 and log k_{ow} observed for the intermediate size class therefore reflects the abrupt changes in growth rate and metabolic demands of reproductive growth and development and compensatory increases in tissue turnover rates. Chemical elimination for these fish reflects growth rate and reproductive demand induced by changes in tissue turnover of the lipid pool.

General relationships of allometry predict limited chemical elimination from tissues in larger, long-lived individuals (Trudel and Rasmussen 1997). Surprisingly, the results of this study demonstrated higher chemical elimination rate constants for the largest individuals (>2500 g). The range in total length for individuals from this size class included fish that typically consume larger, older alewives from the forage base (Jones et al. 1993). With a reduced proportion of these prey in the forage base (Rand et al. 1994), large predators in Lake Ontario faced increased competition for this limited resource and bioenergetics modeling efforts support this conclusion for lake trout 2500 g and larger (He and Stewart 1998). Tissue turnover rates are also predicted to increase as fish grow in size, and increased swimming and foraging activities in a food-limited environment magnify this effect with an increased loss of body energy expected (He and Stewart 1998). The higher k_2 values calculated for lake trout >2500 g are a result of this energetic loss where reliance on endogenous energy reserves resulted in a decrease in lipid content. These kinetic data demonstrate that chemical elimination in lake trout >1500 g is primarily a function of the increased chemical concentration gradients resulting from lipid mobilization to supplement the energetic costs of foraging in a prey-limited environment and the metabolic demands of reproductive development.

The low k_2 values reported for the smallest size class (<1500 g) reflect the lower rates of tissue turnover expected for these individuals that are not food limited. Since this size class is less discriminatory with respect to foraging activities, food limitation is less likely and sufficient prey resources satisfy metabolic requirements and supplement growth. Therefore, limited loss of endogenous reserves is expected than if under conditions of reduced prey densities (Elliot 1976). The extended average PCB $t_{1/2}$ estimate of 33 years calculated for these individuals describes the retention and recycling of endogenous materials into production for the smallest size class as supported by the increased body mass observed for this size class between 1977 and 1993. This contrasts the shorter average PCB $t_{1/2}$ values of 19 and 12 years calculated for the intermediate and largest lake trout size classes whose body mass declined from 1977 to 1993. Lower tissue turnover rates are also predicted for the size range of fish in this size class and minimal endogenous energy loss is predicted by the lower k_2 values determined for these individuals. In contrast with these field observations, elimination rates of persistent pollutants in laboratory fish are negatively correlated with body size (Trudel and Rasmussen 1997). The trend of increased elimination rates in larger lake trout in this study, however, covers less than one order of magnitude in size and is driven by body condition and lipid content of fish. The allometric model describing tissue turnover rates for smaller, immature lake trout is different from that for mature fish predicting tissue turnover rates up to twice those for the larger individuals in this study (He and Stewart 1998). The combined tissue turnover trend from immaturity to maturity therefore follows a general decrease during the life history of a lake trout (He and Stewart 1998), indicating that in situ PCB elimination in mature lake trout is determined by the bioenergetic process

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of lipid turnover. Under this consideration, the low k_2 and extended $t_{1/2}$ values calculated for smaller lake trout appear to reflect a combination of effects including a more robust prey base, lower tissue turnover rates, and resultant chemical concentration gradients that limit the depuration of these chemicals.

Reduced production capacities and conversion efficiencies are predicted for larger size classes of Lake Ontario lake trout principally owing to the declining status of alewife prey (Rand and Stewart 1998*b*). The assimilation of exogenous, or consumed, energy into growth and production has been modeled as energy NCE (He and Stewart 1998). Energy NCEs are predicted to decrease with declines in lake trout growth rate and mass. For larger individuals, the preferred size spectrum of alewife prey increases accordingly (Brandt 1986). Mean masses of older Lake Ontario alewives, however, dropped from ~40 to 20 g during the course of this study (Rand et al. 1994). With larger alewives constituting an increasingly smaller proportion of the preferred forage base, decreased lake trout growth and conversion efficiencies are expected for individuals foraging on less than optimal prey size (Pazzia et al. 2002). The decreasing NCE trend predicted for larger lake trout reflects increased foraging efforts required of these individuals exploiting a suboptimal alewife prey base. To meet metabolic demands in this environment, an increased reliance on endogenous reserves occurs for large lake trout. In this study, the relationship calculated between k_2 and lake trout size describes this increased reliance on endogenous reserves for individuals >1500 g, specifically as the loss of energy due to turnover of the lipid pool.

Production is typically a measure of the assimilation of prey energy into predator growth. The turnover of endogenous reserves, however, can also contribute to production (He and Stewart 1998), but determining the extent of this contribution to growth has not been achieved. The turnover rate of the lipid pool, or endogenous energy use, is hypothesized to be described by the relationship between k_2 and body size. He and Stewart's (1998) modeled specific rate of tissue turnover in eq. 4 describes the mobilization of total body energy, a combination of the lipid and protein reserves. Therefore, the ratio of tissue turnover (τ) to k_2, provides an index of endogenous production or the proportion of tissue turnover that contributes back to production in lake trout. Modeling the ratio τk_2 determined in this study with both lake trout tissue turnover to growth ratios and increasing size produces the same relationships as NCE with these variables. The higher τk_2 ratios predicted for smaller, faster growing lake trout represent a higher contribution of tissue turnover to production than that predicted for larger individuals. Given that these individuals hunt smaller prey, changes in the alewife size spectrum will have less consequence with respect to the energetic demands of foraging. The decreasing trends of τk_2 with both increasing tissue turnover to growth ratios and lake trout size demonstrate an increased loss of endogenous energy reserves as lake trout age, grow in size, but slow down in specific growth rate. The lower τk_2 values for larger individuals also indicate increased endogenous reserve mobilization owing to the increased metabolic demands searching for prey in a food-limited environment.

Modeled gross production of Lake Ontario lake trout has been predicted to decrease in successive year-classes following age 4 at a body size of ~1800 g (Rand and Stewart 1998*b*). This size of lake trout describes the intersection of the curves for the relationships of k_2 and τk_2 with lake trout size. Lake trout size at the intersection of the tissue turnover and NCE functions is slightly larger at ~2100 g. The relationship between k_2 and body size reflects only the rate of lipid turnover, whereas the modeled rate of tissue turnover in eq. 4 describes the turnover rate of total body energy (He and Stewart 1998). As the relationship between k_2 and body size reflects lipid turnover rates, the lower intersection of k_2 with τk_2 at ~1800 g reflects the reliance on lipid reserves as the primary endogenous energy source under less efficient growth conditions. The higher intersection between tissue turnover rates and NCE, however, is likely the result of combined effects of the mobilization of lipid and protein reserves. For larger fish, suitable alewife prey become limited (Rand et al. 1994), endogenous energy demands increase, and lipid reserves are rapidly mobilized as reflected by the higher k_2 values and decreased τk_2 ratios calculated for these fish. In this manner, chemical elimination rates for large predators in this system are primarily influenced by the bioenergetic constraints resulting from food limitation.

The thermodynamic capacity of persistent organic pollutants such as PCBs to reside in lipid stores (Mackay and Paterson 1981) in combination with their refractory nature and general resistance to metabolism (Livingstone 1998) infers the suitability of these compounds to act as surrogate markers of bioenergetic processes. This kinetic approach using persistent chemicals has been demonstrated to provide important interpretation of biomonitoring trend data (Morrison et al. 2002) and is further supported by the results of this research. PCB elimination rates provide in situ measures of tissue turnover rates and can be used to describe the proportion of endogenous energy required to support metabolism and production. Quantifying in situ tissue turnover rates is critical for assessing the bioenergetics of natural populations and is also crucial for understanding relationships between growth, reproduction, and population dynamics (He and Stewart 1998). The integration of chemical concentrations and kinetics into bioenergetics modeling yields a relatively simple, direct, and less resource intensive mechanism to characterize bioenergetic processes in complex ecosystems.

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Bacterial response to dissolved organic matter affects resource availability for algae

Jennifer L. Klug

Abstract: In aquatic systems, the presence of colored dissolved organic matter (DOM) may affect algal growth in numerous ways. This paper focuses on the effects of DOM on resource availability. DOM contains nitrogen and phosphorus, which may become available following microbial or photochemical degradation. Also, addition of DOM may stimulate bacterial growth, which in turn may change the availability of nitrogen, phosphorus, and inorganic carbon to algae. Experiments conducted in a moderately colored lake showed that the effect of DOM on algal growth depended on the amount of nutrients present in the peat extract and on bacterial response to DOM. There was evidence for competition for phosphorus between algae and bacteria in some treatments. In addition, when both bacteria growth and algal growth were high, bacterial respiration of DOM alleviated algal carbon limitation by providing algae with an inorganic carbon source. Thus, the degree to which bacteria are stimulated by the addition of DOM will affect the amount of phosphorus and inorganic carbon available for algal growth. These results suggest that part of the difficulty in predicting algal response to changes in DOM and nutrient concentration may be due partially to variability in bacterial responses.

Résumé : Dans les systèmes aquatiques, la présence de matière organique dissoute (DOM) colorée peut affecter la croissance des algues de diverses manières. Il est question ici des effets de la DOM sur la disponibilité des ressources. La DOM contient de l'azote et du phosphore qui peuvent devenir disponibles par la dégradation microbienne ou photochimique. De plus, l'addition de DOM peut stimuler la croissance bactérienne, qui à son tour peut modifier la disponibilité de l'azote, du phosphore et du carbone inorganique pour les algues. Des expériences menées dans un lac à coloration moyenne montrent que les effets de la DOM sur la croissance des algues dépend de la quantité de nutriments présents dans l'extrait de tourbe et de la réaction des bactéries à la DOM. Il y a des indications qu'il existe une compétition pour le phosphore entre les algues et les bactéries dans certaines des expériences. De plus, quand la croissance des bactéries et celle des algues sont toutes deux élevées, la libération de DOM par la respiration bactérienne diminue la pénurie de carbone pour les algues en leur fournissant une source inorganique de carbone. Ainsi, le degré de stimulation que cause l'addition de DOM chez les bactéries affecte les quantités de phosphore et de carbone inorganique disponibles pour la croissance des algues. Ces résultats laissent croire qu'une partie de la difficulté qu'il y a à prédire la réaction des algues aux changements de DOM et de concentrations de nutriments peut s'expliquer par la variabilité des réactions bactériennes.

[Traduit par la Rédaction]

Introduction

The importance of colored dissolved organic matter (DOM) in freshwater systems has been a topic of limnological research since the late nineteenth century. Early work focused on the concentration of DOM in lakes and used DOM as a means of classifying lakes (Birge and Juday 1927; Strom 1928). However, modern work has shown that DOM concentration may have high intra- and inter-annual variability as a result of natural causes and human activities (e.g., D.W. Schindler et al. 1997). Other studies have proposed that humic lakes may respond differently than clear-water lakes to large-scale human perturbations such as acidification, eutrophication, and increased ultraviolet radiation (e.g., DeHaan 1992), which suggests fundamental differences between these two classes of lakes.

Colored DOM is a mixture of organic compounds, including humic and fulvic acids, which usually enter the lake from terrestrial sources through surface flow and groundwater (McKnight and Aiken 1998). Organic matter is also produced within lakes via algal excretion, but this organic matter is not colored, has lower molecular weight, and is generally more degradable than organic matter produced outside the lake (McKnight and Aiken 1998). High concentrations of DOM give lakes an orange-brown color and have diverse effects on the physical and chemical structure of the water column (see Hessen and Tranvik (1998) and Williamson et al. (1999) for reviews of the role of DOM in lakes). The light-absorbing properties of DOM greatly reduce the depth to which light penetrates into the water column, leading to less photosynthetically active light available for photosynthesis (Jones 1992; Carpenter et al. 1998) and less potentially damaging ultraviolet radiation (Morris et al. 1995). The presence of DOM may also alter the availability of nutrients to algae (Jones 1998). DOM is

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a mixture of organic compounds containing phosphorus and nitrogen (Jansson 1998). The bioavailability of these nutrients is not well known, but it is likely that at least some of the nutrients are available to algae (Arvola et al. 1996). In addition, phosphorus and nitrogen in humic matter may become available through bacterial decomposition and photochemical transformations (Carlsson and Graneli 1993; Vähätalo et al. 2003). The relative magnitude of the nutrient vs. shading effects is a function of the nutrient characteristics and light-absorbing properties of different sources of DOM (Klug 2002). For example, the composition and proportion of fulvic acid, a component of DOM that absorbs visible and ultraviolet radiation (McKnight and Aiken 1998), varies across DOM source.

Studies have shown that the algal communities in lakes with high concentrations of DOM respond less strongly to the addition of inorganic nutrients (nitrogen and phosphorus) than those in clear-water lakes (Chow-Fraser and Duthie 1987; Carpenter et al. 1998; Klug and Cottingham 2001). There are several mechanisms by which this could occur. One explanation is that added phosphorus adsorbs to DOM and is unavailable to phytoplankton (DeHaan 1992). A second hypothesis is that DOM reduces pH, which may adversely affect cyanobacteria (Klug and Cottingham 2001). Cyanobacteria, which are often responsible for increases in biomass following nutrient addition, are not as likely to bloom in lakes with high DOM (Chow-Fraser and Duthie 1987; Klug and Cottingham 2001). A third explanation is that low light availability due to high levels of DOM may prevent algae from responding strongly to increases in nutrient concentration (Carpenter et al. 1998). A fourth hypothesis is that high levels of DOM may alter the interactions between algae and bacteria such that nutrients such as phosphorus are sequestered by bacteria and thus less available to algae (Stewart and Wetzel 1982; Jansson et al. 1999).

The interactions between algae and bacteria often seem paradoxical, because studies have shown that bacteria are superior competitors for phosphorus (Currie and Kalff 1984) and should outcompete algae. However, algae and bacteria coexist in all lakes. One explanation for algae–bacteria coexistence is that algae are nutrient-limited and bacteria are energy-limited (i.e., carbon-limited). In many systems, the energy needs of bacteria are met by extracellular release of organic carbon by algae. As bacteria sequester phosphorus, algal production (and thus carbon excretion) may decrease because of the reduction in phosphorus availability. The reduction in carbon excretion may decrease bacterial production, which leads to more phosphorus available for algae (Jansson 1998). However, in many lakes, bacteria may be nutrient-limited rather than energy-limited (Le et al. 1994; Elser et al. 1995). In high DOM lakes in particular, bacteria may use DOM as a carbon source, which reduces or eliminates their dependence on algal carbon (Hessen 1992). If bacteria are nutrient-limited (Jansson et al. 1996), they will be in direct competition with algae for mineral nutrients such as phosphorus (Bratbak and Thingstad 1985; Jansson et al. 1999; Drakare et al. 2002). Thus, if high levels of DOM stimulate bacterial growth, nutrient availability for algae may be reduced because bacteria are superior competitors for phosphorus (Currie and Kalff 1984; Jansson 1998). In addition, if high levels of DOM stimulate bacterial growth, respiration of DOM by bacteria may provide algae with an inorganic carbon source. In some lakes, supersaturated levels of inorganic carbon are sustained by bacterial respiration of DOM (Cole et al. 2000).

To explore how interactions between algae and bacteria affect algal response to changes in nutrients and DOM, I manipulated combinations of inorganic nutrients, DOM, and simple organic and inorganic carbon sources in short-term experiments. Because I expected competition for phosphorus to be important and competition for phosphorus to depend on carbon availability, I manipulated sources of nutrients and carbon that were differentially available to algae and bacteria: inorganic nutrients are available to both algae and bacteria; DOM provides a complex source of carbon and nutrients with unknown availability; glucose is a simple organic carbon source available to bacteria; and bicarbonate is an inorganic carbon source available to algae.

Materials and methods

Study site

West Long Lake is a 3.5 ha, moderately colored seepage lake located in Gogebic County, Michigan (89°32′W, 46°13′N), at the University of Notre Dame Environmental Research Center (UNDERC). As part of a whole-lake experiment designed to test whether lakes with different food webs responded differently to nutrient addition, West Long Lake was fertilized with nitrogen and phosphorus from 1993 to 1997 (summarized in Carpenter et al. 2001). The N:P ratio of the whole-lake fertilization was chosen to maintain P limitation in West Long Lake (Carpenter et al. 2001). I conducted experiments in the lake in 1998 and 1999. Because of possible nutrient carryover from the whole-lake experiment and because lakes in this region tend to be P-limited (Elser et al. 1988), I designed my enrichment treatments to maintain P limitation of phytoplankton. Limnological conditions in West Long Lake at the time of the experiments are provided (Table 1).

Experimental design

To test my hypotheses concerning the effects of DOM and nutrients on algal growth, I conducted two short-term experiments in 10-L mesocosms (Table 2). The first experiment was designed to test whether DOM extracted from *Sphagnum* peat moss affected algal growth. Three replicates of four treatments were prepared: control; NP (inorganic nitrogen (potassium nitrate) and phosphorus (sodium phosphate) added at a N:P ratio of 23:1 by atoms); DOM (extracted from freshly collected peat moss); and NPDOM (NP and DOM). The experiment was conducted from 11 to 17 August 1998. Response variables were algal biomass (estimated as chlorophyll *a* concentration) and bacterial production.

The second experiment tested the response of algae and bacteria to different carbon sources. Three carbon sources were used: DOM from freshly collected peat; glucose (C increase from glucose = 12.7 mg $C \cdot L^{-1}$); and bicarbonate. Bicarbonate was added as sodium bicarbonate and increased dissolved inorganic carbon (DIC) by 1000 μg $C \cdot L^{-1}$. Addition of bicarbonate raised pH by ~0.2 pH units. Addition of peat moss extract lowered pH by ~0.5 pH units.

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Table 1. Dissolved organic carbon (DOC), total phosphorus (TP), phosphate, total nitrogen (TN), nitrate, and ammonium concentrations in West Long Lake at the time of the experiments and in the peat extract.

	pH	Water color, g_{440} (m^{-1})	DOC (mg C·L^{-1})	DIC (mg C·L^{-1})	TP (μg P·L^{-1})	Phosphate (μg P·L^{-1})	TN (μg N·L^{-1})	Nitrate (μg N·L^{-1})	Ammonia (μg N·L^{-1})
West Long Lake: late July 1998	5.9	4.5	10.2	0.813	27.8	2.31	690.2	2.25	13.8
West Long Lake: June 1999	6.3	4.4*	9.3	0.698	20.6	1.7	620.1	10.6	22.7
Peat extract: 1998	na	23.2	89.3	na	178.3	152.5	2217.4	20.4	451.1
Peat extract: 1999	4.4	21.1	50.0	4.2	186.8	146.2	1909.2	31.4	41.2

Note: Data not available are denoted as na. g_{440}, measure of water color based on absorbance at 440 nm.
*Annual average for 1999.

Table 2. Dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total phosphorus (TP), total nitrogen (TN), and the percentage of the added nutrients that were added in inorganic form for each experimental treatment.

Experiment	Treatment	DOC added (mg C·L^{-1})	DIC added (mg C·L^{-1})	Total P added (μg P·L^{-1})	Inorganic P added (% of total P)	Total N added (μg N·L^{-1})	Inorganic N added (% of total N)
1	Control	0	0	0	0	0	0
	NP	0	0	32	100	320	100
	DOM	8.9	na	17.8	86	219.7	21
	NPDOM	8.9	na	49.8	95	539.7	68
2	Control	0	0	0	0	0	0
	NP	0	0	32	100	320	100
	DOM	5.0	0.42	18.7	78	190.9	4
	Glucose	12.7	0	0	0	0	0
	Bicarbonate	0	1.0	0	0	0	0
	NPDOM	5.0	0.42	50.7	92	510.9	64
	NPG	12.7	0	32	100	320	100
	NPBC	0	1.0	32	100	320	100

Note: NP, added nitrogen and phosphorus; DOM, added dissolved organic matter (DOM); NPDOM, added nitrogen, phosphorus, and DOM; NPG, added nitrogen, phosphorus, and glucose; NPBC, added nitrogen, phosphorus, and bicarbonate. Data not available are denoted as na.

These carbon sources were added separately and in combination with NP with three replicates per treatment for a total of eight treatments. The experiment was conducted from 23 to 28 June 1999. Response variables were algal biomass (estimated as chlorophyll *a* concentration), bacterial production, and DIC.

Experimental protocol

An integrated sample of the epilimnion was used in the experiments. To avoid light damage to phytoplankton, water was collected between 0730 and 0900. Water was passed through an 80-μm-mesh net to remove cladocerans, copepods, and most rotifers. Although very large phytoplankton were also excluded, they averaged less than 3.1% of total algal biovolume (S.R. Carpenter, Center for Limnology, University of Wisconsin, 680 North Park Street, Madison, WI 53706-1492, USA, unpublished data). Ciliates and other heterotrophic protozoans passed through the mesh (J.L. Klug, personal observation). Treatments (see Experimental design) were mixed in 100-L plastic pails and then poured into 10-L polyethylene square mesocosms (I-Chem, Chase Scientific Glass, Inc., Rockwood, TN 37854). The mesocosms were attached to a floating wooden frame and incubated in situ. All replicates were incubated 0.75 m below the water surface, which corresponded to ~25% of surface PAR (photosynthetically active radiation). Although there were visible differences in color between the treatments with and without peat extract, shading was similar in all treatments because the containers were small enough that light availability was determined by the depth of the mesocosm in the water column rather than by the concentration of DOM within the mesocosm.

Fresh peat extract was collected from a depth of 25 cm in the bog mat surrounding Tuesday Lake, a small lake less than 5 km from West Long Lake. Liquid was squeezed from the peat by hand and returned to the lab. The fresh peat extract was coarse-filtered through a muslin bag, centrifuged for 10 min at 7000 rpm, filter-sterilized through either an 0.2-μm or 0.45-μm filter cartridge (Whatman Inc., Florham Park, NJ 07932) to remove bacteria, refrigerated, and used within 2 days. I measured DOC concentration of the extract using an O/I TOC analyzer (O/I Analytical, College Station, TX 77842) with persulfate oxidation and phosphorus and nitrogen concentrations using an autoanalyzer (Lachatt Instruments, Milwaukee, WI 53218). DOC and nutrient concentrations differed among extracts, which led to different concentrations added to each experiment (Tables 1, 2).

Biological and chemical analyses

Chlorophyll *a* concentration was determined fluorometrically to assess changes in total algal biomass. Samples (100–200 mL) were filtered onto a Whatman GF/F filter, frozen,

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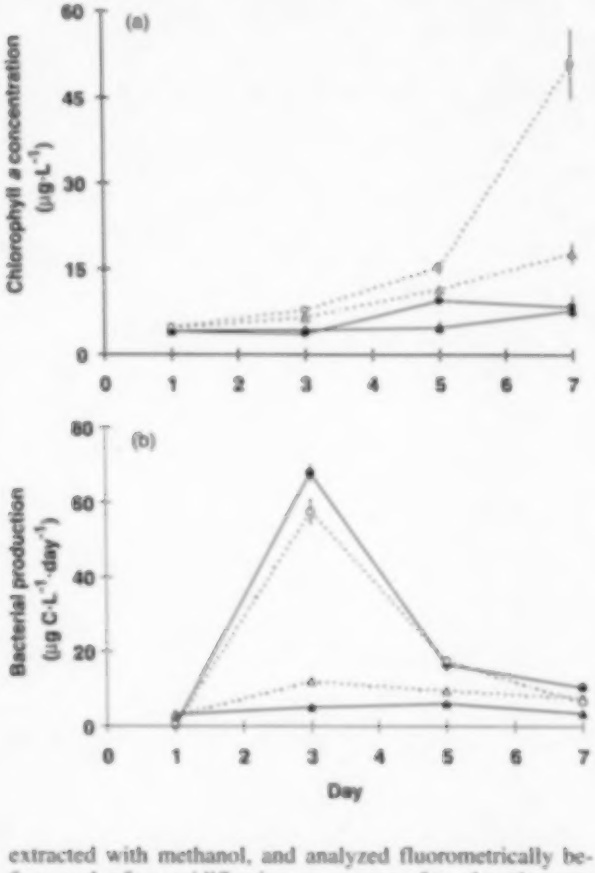
Fig. 1. Means and standard errors of (*a*) chlorophyll *a* concentration and (*b*) bacterial production throughout experiment 1 (—▲—, the control treatment; - -△- -, the added nitrogen and phosphorus (NP) treatment; —●—, the added dissolved organic matter (DOM) treatment; - -○- -, the treatment with added NP and DOM).



extracted with methanol, and analyzed fluorometrically before and after acidification to correct for pheopigments (Marker et al. 1980). Bacterial net production was estimated using the microcentrifuge ^{3}H-leucine method for measuring protein production (Smith and Azam 1992), as modified in Bade et al. (1998). pH was measured using an electrode that was presoaked in sample water to obtain stable readings. DIC was measured using a Shimadzu GC-8AIT gas chromatograph (Shimadzu America / Shimadzu Scientific Instruments, Inc., Columbia, MD 21046) (Stainton 1973).

Statistical analyses

I used repeated-measures analysis of variance (RM-ANOVA) to assess time effects, treatment effects (DOM, NP, glucose, and (or) bicarbonate effects), and time × treatment interactions on chlorophyll *a* concentration and bacterial production (Gurevitch and Chester 1986). Data were transformed logarithmically to normalize the data and homogenize variances. The chlorophyll *a* data and some of the bacterial production data did not meet the assumption of sphericity of the covariance matrix required by univariate RM-ANOVA; therefore, I used multivariate RM-ANOVA as suggested in Potvin et al. (1990). Departure from sphericity is common in time series data because adjacent time points are more highly correlated than distant time points (Von Ende 1993).

Table 3. Chlorophyll *a*: repeated-measures analysis of variance results for experiment 1.

	Treatment effects			
Source	MS	df	*F*	$p > F$
DOM	0.93	1	104.64	0.0001
NP	5.46	1	614.67	0.0001
DOM × NP	0.17	1	19.38	0.0023
Error	0.07	8		
	Time and time × treatment effects			
Source	*F*	Num df	Den df	$p > F$
Time	720.95	3	6	0.0001
Time × DOM	63.20	3	6	0.0001
Time × NP	127.89	3	6	0.0001
Time × DOM × NP	17.34	3	6	0.0023

Note: DOM, main effect of added dissolved organic matter; NP, main effect of added nitrogen and phosphorus; DOM × NP, interaction between the main effects. Num, numerator; Den, denominator.

Results

Experiment 1

The first experiment was designed to test the effects of DOM on algal growth. Algal biomass (estimated as chlorophyll *a* concentration) was higher in the DOM (added dissolved organic matter) and NP (added nitrogen and phosphorus) treatments than in the control treatment (Fig. 1*a*; Table 3). Algal response to nutrients was enhanced by the presence of DOM as revealed by the DOM × NP interaction (Fig. 1*a*; Table 3).

Bacterial production increased in the NP, DOM, and NPDOM treatments (Fig. 1*b*; Table 4). The magnitude of the bacterial response in the NPDOM treatment was similar to the response to DOM alone (Fig. 1*b*; Table 4). Thus, although there is a significant NP × DOM interaction (Table 4), the direction is opposite that of the algae (Fig. 1). That is, the bacterial response in the NPDOM treatment was less than expected based on the response to DOM and NP alone. Bacterial production peaked several days earlier than the algal response, suggesting that bacteria were responding to the experimental treatments rather than to the changes in algal biomass (Fig. 1).

Experiment 2

I conducted experiment 2 to explore whether the results from experiment 1 could be explained by interactions between algae and bacteria. I measured algal and bacterial response to three carbon sources (glucose, bicarbonate, and DOM) alone and in combination with NP to test (*i*) whether competition between algae and bacteria for phosphorus affected algal growth and (*ii*) whether bacterial respiration of DOM provided phytoplankton with an inorganic carbon source. The DOM treatments provide complex carbon and nutrient sources, whereas glucose provides a readily used or-

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Table 4. Bacterial production: repeated-measures analysis of variance results for experiment 1.

	Treatment effects			
Source	MS	df	*F*	$p > F$
DOM	2.21	1	85.82	0.0001
NP	0.65	1	25.29	0.001
DOM × NP	0.96	1	37.52	0.0003
Error	0.21	8		
	Time and time × treatment effects			
Source	*F*	Num df	Den df	$p > F$
Time	1463.9	3	6	0.0001
Time × DOM	562.28	3	6	0.0001
Time × NP	3.45	3	6	0.092
Time × DOM × NP	44.80	3	6	0.0002

Note: DOM, main effect of added dissolved organic matter; NP, main effect of added nitrogen and phosphorus; DOM × NP, interaction between the main effects.

Table 5. Chlorophyll *a*: repeated-measures analysis of variance results for experiment 2.

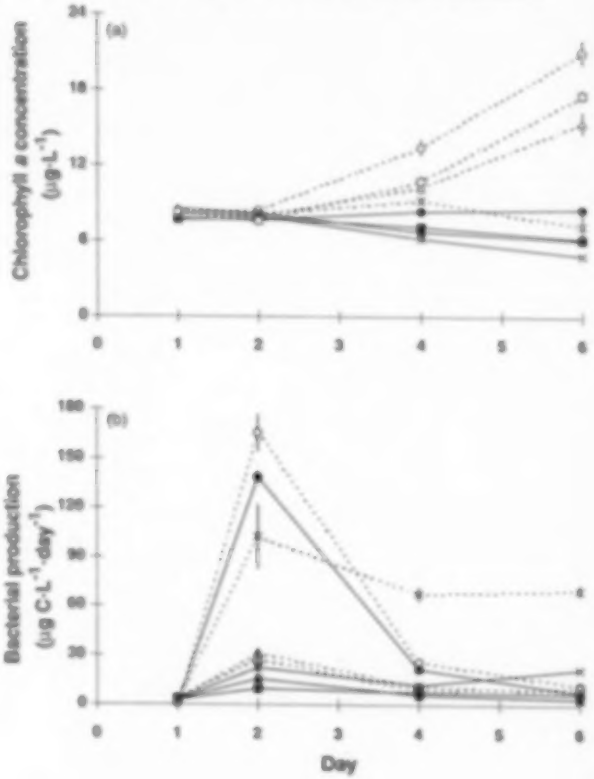
	Treatment effects			
Source	MS	df	*F*	$p > F$
DOM	0.20	1	40.34	0.0001
NP	0.61	1	125.41	0.0001
Glucose	0.31	1	63.04	0.0001
Bicarbonate	0.0005	1	0.11	0.75
DOM × NP	0.009	1	1.90	0.19
NP × glucose	0.042	1	8.49	0.01
NP × bicarbonate	0.008	1	1.70	0.21
Error	0.004	16		
	Time and time × treatment effects			
Source	*F*	Num df	Den df	$p > F$
Time	15.90	3	14	0.0001
Time × DOM	34.76	3	14	0.0001
Time × NP	31.75	3	14	0.0001
Time × glucose	15.43	3	14	0.0001
Time × bicarbonate	4.25	3	14	0.025
Time × DOM × NP	0.49	3	14	0.70
Time × NP × glucose	4.90	3	14	0.016
Time × NP × bicarbonate	4.41	3	14	0.022

Note: DOM, main effect of added dissolved organic matter; NP, main effect of added nitrogen and phosphorus.

ganic carbon source for bacteria, and bicarbonate provides an inorganic carbon source for algal photosynthesis.

Algal biomass increased relative to the control in all of the treatments in which nutrients were added (Fig. 2*a*; Table 5). Averaged across time, there were positive effects of DOM and NP and a negative effect of glucose, as well as an NP × glucose interaction (Fig. 2*a*; Table 5). In addition, the analyses suggest that many of the manipulated factors had significantly different trajectories through time. There was a time × treatment interaction for DOM, NP, glucose, and bicarbonate, as well as NP × glucose (NPG) and NP × bicarbonate (NPBC) interactions (Table 5). The latter interactions show that the algae responded differently to nutrients in the presence of added carbon. Algal response to nutrients was enhanced in the presence of bicarbonate and depressed in the presence of glucose (Fig. 2*a*).

Fig. 2. Means and standard errors for (*a*) chlorophyll *a* concentration and (*b*) bacterial production throughout experiment 2 (—▲—, the control treatment; - -△- -, the added nitrogen and phosphorus (NP) treatment; —●—, the added dissolved organic matter (DOM) treatment; - -○- -, the treatment with added NP and DOM; —×—, the added glucose treatment; —■—, the added bicarbonate treatment; - -◈- -, the added NP and glucose treatment; - -□- -, the added NP and bicarbonate treatment).



Bacterial production increased strongly in the DOM, NPDOM, and NPG treatments (Fig. 2*b*). Weaker positive responses were observed in the NP (alone) and glucose (alone) treatments (Fig. 2*b*). Averaged across time, there were significant effects of DOM, NP, glucose, and a NPG interaction (Table 6). As in the algal responses, bacterial production responded to the manipulated factors differently across time. There were significant time × treatment interactions for DOM, NP, glucose, and bicarbonate, as well as interactions between all carbon sources and NP (Table 6). The presence of these interactions suggests that, across time, bacteria respond differently to nutrients depending on the carbon source. Bacterial response to nutrients is enhanced in the presence of organic carbon sources (DOM and glucose) and

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Table 6. Bacterial production: repeated-measures analysis of variance results for experiment 2.

	Treatment effects			
Source	MS	df	F	$p > F$
DOM	4.31	1	68.67	0.0001
NP	2.66	1	42.35	0.0001
Glucose	11.0	1	175.24	0.0001
Bicarbonate	0.03	1	0.40	0.54
DOM × NP	0.11	1	1.75	0.21
NP × glucose	1.15	1	18.34	0.0006
NP × bicarbonate	0.003	1	0.05	0.85
Error	1.0	16		
	Time and time × treatment effects			
Source	F	Num df	Den df	$p > F$
Time	551.14	3	14	0.0001
Time × DOM	166.39	3	14	0.0001
Time × NP	5.45	3	14	0.011
Time × glucose	104.05	3	14	0.0001
Time × bicarbonate	7.24	3	14	0.004
Time × DOM × NP	6.06	3	14	0.007
Time × NP × glucose	6.81	3	14	0.005
Time × NP × bicarbonate	5.19	3	14	0.013

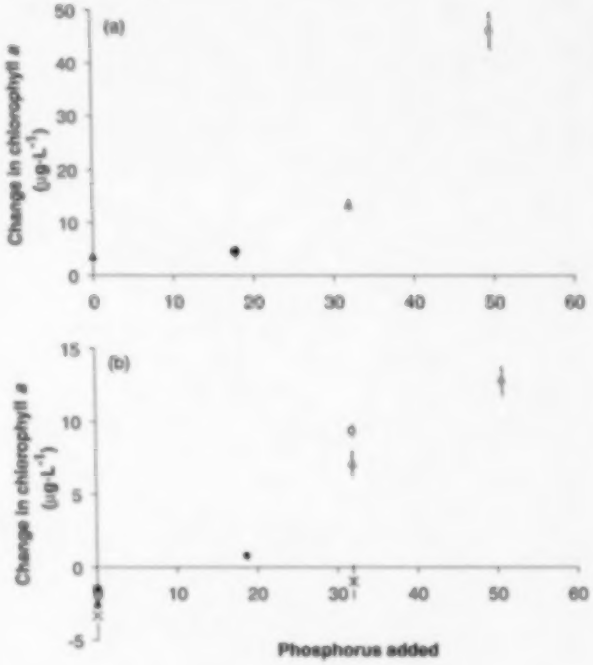
Note: DOM, main effect of added dissolved organic matter; NP, main effect of added nitrogen and phosphorus.

slightly depressed in treatments with added bicarbonate (Fig. 2*b*).

Interactions between algae and bacteria

In general, there was positive relationship between overall algal growth and the concentration of phosphorus added to each treatment as fertilizer or as part of the peat extract (Fig. 3). However, differences between algal growth among treatments with similar added-phosphorus concentrations suggest that competition between algae and bacteria was important and that bacterial response to additions of organic carbon affected phosphorus availability for algae. In experiment 2, in the three treatments with no added phosphorus (control, glucose, bicarbonate), the pattern of algal growth (bicarbonate > control > glucose) is opposite that of bacterial production (glucose > control > bicarbonate). In these treatments, phosphorus is likely limiting and there may be strong competition between algae and bacteria for that resource. There was also variability in algal growth among the NP, NPBC, and NPG treatments, which all had the same concentration of added phosphorus (Fig. 3*b*). The much lower growth in the NPG treatment relative the NP treatment is likely due to bacterial uptake of the added phosphorus as bacterial production in the NPG treatment was high throughout the entire experiment (Fig. 2*b*). The higher growth in the NPBC treatment relative to the NP treatment is expected if algae in the NP treatment became carbon-limited. The inorganic carbon added in the NPBC treatment would have alleviated this limitation and resulted in higher growth. In addition, comparison of algal growth in the DOM and NPDOM treatments between experiments suggests that bacterial response to DOM may change nutrient availability for

Fig. 3. Means and standard errors for the change in chlorophyll *a* concentration over the course of the experiment vs. the amount of phosphorus added in (*a*) experiment 1 and (*b*) experiment 2 (▲, the control treatment; △, the added nitrogen and phosphorus (NP) treatment; ●, the added dissolved organic matter (DOM) treatment; ○, the treatment with added NP and DOM; ×, the added glucose treatment; ■, the added bicarbonate treatment; ⊛, the added NP and glucose treatment; □, the added NP and bicarbonate treatment).



algae. The peat extract in both experiments contained similar amounts of phosphorus, yet algal growth was higher in both the DOM and NPDOM treatments in experiment 1 than in the same treatments in experiment 2. Again, this pattern is opposite to that of the bacterial response. Peak bacterial production in the DOM and NPDOM treatments was approximately twice as high in experiment 2 relative to experiment 1 and would have resulted in higher phosphorus uptake by bacteria.

When both DOM and NP were added to the mesocosms, both algae and bacteria responded positively (Figs. 1, 2). In experiment 1, there was a strong NP × DOM interaction, suggesting that nutrients alone were not driving this relationship. One hypothesis is that the NP × DOM interaction was due to algal use of DIC respired by bacterial degradation of the added DOM. Changes in DIC throughout experiment 2 support this hypothesis. At the beginning of experiment 2, initial DIC concentration was higher in treatments with added DOM (because of the DIC present in the extract) and bicarbonate than the other treatments (Fig. 4). By the end of the experiment, DIC concentration had decreased in all treatments with added nutrients, presumably because of uptake by rapidly growing algae. The exception to this pattern was the NPG treatment, where algae did not grow well and DIC

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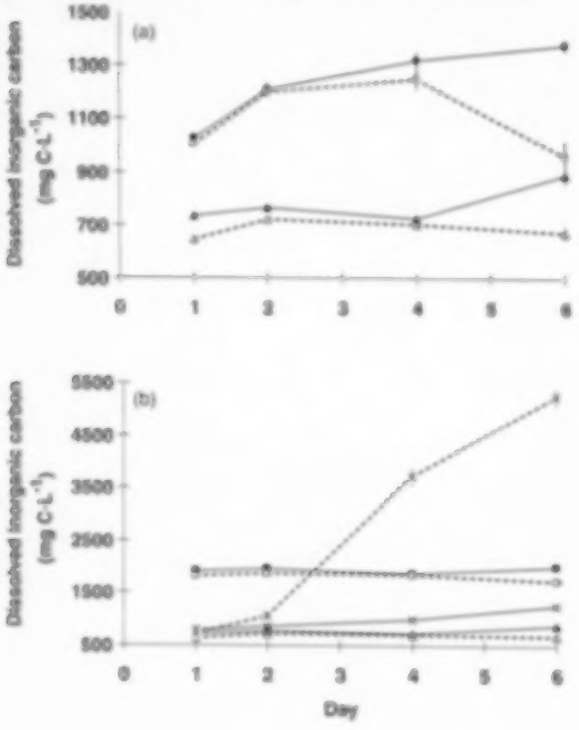
Fig. 4. Means and standard errors for dissolved inorganic carbon in experiment 2 for (*a*) the control, added nitrogen and phosphorus (NP), and added dissolved organic matter (DOM) treatments and (*b*) the control, added NP, added glucose, added bicarbonate, added NP and glucose, and added NP and bicarbonate treatments (—▲—, the control treatment; - -△- -, the added nitrogen and phosphorus (NP) treatment; —●—, the added dissolved organic matter (DOM) treatment; - -○- -, the treatment with added NP and DOM; —×—, the added glucose treatment; —■—, the added bicarbonate treatment; - -⊕- -, the added NP and glucose treatment; - -□- -, the added NP and bicarbonate treatment).

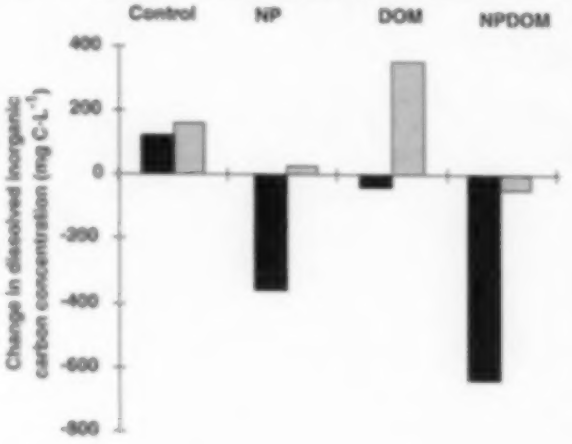


increased throughout the course of the experiment (Fig. 4*b*). In treatments in which algae grew well, I calculated the expected DIC at the end of the experiment by subtracting the amount of carbon sequestered in algal biomass (assuming a chlorophyll *a* : carbon ratio of 1:50 (midrange of values given in Harris 1986)) from the initial DIC concentration (Fig. 5). The discrepancy between observed vs. expected DIC suggests that algae used an inorganic carbon source other than the initial DIC. This additional carbon source likely originated as DOM, which was taken up by bacteria. This assimilated carbon was then either respired by bacteria or by protozoans that consumed the bacteria.

Discussion

To explore how interactions between algae and bacteria affect algal response to changes in nutrients and DOM, I manipulated nitrogen, phosphorus, and carbon sources differentially available to the two groups. Variation in algal growth among treatments appeared to be due to changes in phosphorus and inorganic carbon availability.

Fig. 5. Expected change (solid bars) in dissolved inorganic carbon (initial DIC concentration – carbon in algal biomass) and observed change (shaded bars) in DIC for selected treatments in experiment 2. NP, added nitrogen and phosphorus; DOM, added dissolved organic matter; NPDOM, added NP and DOM.



Responses to DOM

DOM extracted from fresh peat moss contained nitrogen and phosphorus that were available for uptake by algae and bacteria, and it is likely that an increase in nutrients was one mechanism responsible for the increase in algal and bacterial growth in the treatments with added DOM. Both algae and bacteria were stimulated by the addition of inorganic nitrogen and phosphorus, suggesting that they were nutrient-limited at the time of the experiments. Bacterial response in the DOM treatment was similar to that in the NPDOM treatment and also positive in the treatments with added glucose, suggesting that bacteria were co-limited by carbon and nutrients. There are a number of other mechanisms by which DOM may affect aquatic organisms (*e.g.*, by changing pH, absorbing ultraviolet radiation, and changing availability of trace minerals), which are beyond the scope of this manuscript (see Hessen and Tranvik (1998) and Williamson et al. (1999) for reviews of the role of DOM in lakes).

Interactions between nutrients and carbon

Interactions between added nutrients (NP) and various sources of carbon on algal growth were observed in both experiments. In experiment 1, algal biomass was higher than expected in the NPDOM treatment. In experiment 2, algal biomass was higher than expected in the NPBC treatment and lower than expected in the NPG treatment. When nutrients were added to the closed containers, algal growth may have switched from nutrient to carbon limitation. This carbon limitation may have been alleviated in treatments with added DOM, where some DIC was added with the DOM extract and presumably bacterial respiration of DOM provided carbon dioxide (experiment 1), or in treatments with added bicarbonate, where inorganic carbon was added directly (experiment 2). The alleviation of carbon limitation would lead to the observed positive NP × DOM and NP × bicarbonate interactions. In contrast, the negative NP × glucose interaction for algal growth can be explained by competition with

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bacteria for phosphorus. Bacterial production was high when both nutrients and glucose were added, and bacteria may have sequestered much of the phosphorus under these conditions. There was no NP × DOM interaction for algal growth in experiment 2. One explanation is that, for algae, the effects of reduced carbon limitation were offset by competition for phosphorus with bacteria. Bacterial production was much higher in the NPDOM treatment in experiment 2 than in experiment 1. The explanations of the mechanisms leading to the observed interactions are supported by studies in other humic lakes. In Sweden, Drakare et al. (2002) showed that inputs of high DOM river water stimulated bacterial production and that bacteria outcompeted algae for inorganic nutrients following these high-flow events. Similarly, studies have found reduced algal growth in experiments in which both glucose and inorganic nutrients were added and hypothesize that their results were due to bacterial sequestration of nitrogen and phosphorus (Järvinen and Salonen 1998; Joint et al. 2002).

Previous work has established that algae in temperate lakes are typically phosphorus-limited (e.g., Schindler 1977; Shapiro 1988). In addition, nutrient-limitation experiments conducted in lakes near West Long Lake have concluded that algae are phosphorus-limited when the zooplankton community is dominated by large cladocerans, as was the case in West Long Lake (Elser et al. 1988). Järvinen and Salonen (1998) found the same pattern of phosphorus limitation when a humic lake in Finland was dominated by large cladocerans. The experiments described in this paper were designed to maintain phosphorus limitation, although it is possible that the large additions of nitrogen and phosphorus together may have led to co-limitation by nitrogen and phosphorus. Other results from humic lakes suggest that nitrogen limitation of phytoplankton can occur when large cladocerans are absent (Järvinen and Salonen 1998) or when DOM concentrations are higher than those present in West Long Lake (Jansson et al. 2001). Many lakes in the West Long Lake region are softwater lakes with relatively low pH and low concentrations of DIC, even when carbon dioxide is supersaturated. During periods of high algal growth, some lakes are undersaturated and pull carbon dioxide from the atmosphere (D.E. Schindler et al. 1997). In contrast, lakes with high levels of DOM are often supersaturated with respect to inorganic carbon. Supersaturation may be due to bacterial respiration of the allochthonous carbon source (del Giorgio et al. 1999; Cole et al. 2000). Cole et al. (2000) showed that high DOM lakes remain supersaturated, even during periods of high algal growth. Thus, bacterial respiration of DOM could prevent short-term algal carbon limitation during periods of high growth by providing algae with an inorganic carbon source. In some cases, photochemical oxidation of DOM can also satisfy a portion of algal carbon demand (Vähätalo et al. 2003) but was unlikely to be an important mechanism in the experiments described in this paper because ultraviolet radiation was attenuated before it reached the depth at which the mesocosms were incubated (Williamson et al. 1996).

Effects of DOM source

The mechanisms by which DOM affects algal growth and the interactions between algae and bacteria will likely depend on the source of the DOM. This paper considers DOM extracted from peat moss, which contained relatively high nutrient concentrations and was readily used by bacteria. Previous studies using extracts from peat moss or bogs in other regions have also seen an increase in nutrient concentration in DOM treatments (Guildford et al. 1987; Arvola et al. 1996). Other sources of DOM have different properties. For example, Tulonen et al. (1992) found that high molecular weight size fractions of DOM contained higher concentrations of phosphorus than lower molecular weight fractions. This means that phosphorus availability may be different in sources of DOM that contain different compounds. Similarly, the availability of carbon to bacteria may vary across DOM sources. For example, Moran and Hodson (1990) showed that DOM extracted from a lake was more bioavailable to bacteria than DOM extracted from a blackwater marsh.

Role of protozoan grazers

Ciliate and heterotrophic nanoflagellate (HNF) protozoans play an important role in aquatic food webs through bacterivory and nutrient recycling (Fenchel 1987), and those <80 μm were present in these experiments. Previous research has shown that these organisms suffer high mortality from grazing by *Daphnia* (Pace and Funke 1991; Pace and Vaqué 1994), and macrozooplankton-removal experiments have shown an increase in ciliate biomass in less than 1 week (Ventelä et al. 2002), which is the same time scale as the experiments presented in this paper. The zooplankton community in West Long Lake was dominated by *Daphnia* at the time of the experiments. *Daphnia* were excluded from the mesocosms, and this exclusion may have resulted in an increase in ciliates and HNFs. It is impossible to determine how this increase may have affected the outcome of the experiments. If the protozoan grazers were purely regulated by their predators, then the increase should have been similar in all treatments, which should have had little effect on the observed differences in treatments. If the protozoan grazers also responded to changes in a main food source (bacteria), then protozoan grazing and nutrient recycling may have been greater in treatments with higher bacterial production, which may have increased nutrient availability to algae in those treatments. However, algal growth was very low in the treatment with the highest sustained bacterial production (NPG), which suggests that the nutrients in that treatment were sequestered by bacteria and not recycled through protozoan grazing.

Algal community composition

The experiments presented here do not address how differences in algal community composition could affect the outcome of the interactions described above. The algal community in West Long Lake at the time of the experiments was dominated by cryptophytes (J.L. Klug, unpublished data). Several cryptophyte characteristics make them well suited to colored lakes. Cryptophytes are able to survive at low light levels (Klaveness 1988) and may undergo diurnal vertical migration from the epilimnion to the nutrient-rich hypolimnion (Salonen et al. 1984). In addition, there is evidence that some cryptophyte species are mixotrophic and may consume bacteria (Tranvik et al. 1989). Although the

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magnitude of mixotrophy is difficult to quantify in the field, it has been proposed that algae may reduce competition for phosphorus by consuming bacteria, thereby fulfilling their phosphorus requirements with bacterial biomass (Nygaard and Tobiesen 1993; Jansson et al. 1996). It is unlikely that mixotrophy could account for the positive effects of DOM on algae in this study. Although cryptophytes were the dominant taxa at the beginning of the experiment, most taxa (including obligate autotrophs such as chlorophytes) were stimulated by additions of DOM. In addition, algal biomass was low in the treatment with the highest bacterial production, suggesting that algae were in direct competition with bacteria for phosphorus and were not able to escape competition by consuming bacteria.

In conclusion, the results suggest that the effects of changes in nutrients and DOM on algal growth were partially mediated by competition with bacteria for phosphorus. When bacterial production was high and nutrients were limiting, fewer nutrients were available for algal growth. The importance of the competitive interaction will depend on how much DOM stimulates bacterial production and the ambient concentration of nutrients available to bacteria and algae. These characteristics vary according to the source of DOM in lakes. Thus, future work on the relationship between DOM and algae should focus on ways of predicting the algal and bacterial availability of resources of different sources of DOM (e.g., Shaw et al.'s (2000) study on the mechanisms of phosphate–iron–DOM associations and Crump et al.'s (2003) study of seasonal changes in bacterial populations resulting from seasonal changes in DOM source).

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ERRATUM / ERRATUM

Erratum: Fish community change in Lake Superior, 1970–2000

Charles R. Bronte, Mark P. Ebener, Donald R. Schreiner, David S. DeVault, Michael M. Petzold, Douglas A. Jensen, Carl Richards, and Steven J. Lozano

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In the caption of Fig. 6, species names were incorrectly associated to the figure panels. The correct associations are as follows:

Fig. 6. Biomass of lake whitefish (*Coregonus clupeaformis*) (*a*), bloater (*Coregonus hoyi*) (*b*), kiyi (*Coregonus kiyi*) (*c*), ninespine stickleback (*Pungitius pungitius*) (*d*), burbot (*Lota lota*) (*e*), slimy sculpin (*Cottus cognatus*) (*f*), spoonhead sculpin (*Cottus ricei*) (*g*), and deepwater sculpin (*Myoxocephalus thompsoni*) (*h*) in U.S. (□) and Canadian (●) waters determined by lakewide bottom trawl surveys in nearshore waters (15–100 m), 1978–1999 conducted by the U.S. Geological Survey.

Similarly, various references to specific figure panels in the text need to be corrected as follows:

(*i*) On page 1561, 2nd column, under Other forage species, line 4 should read "... averaged about 0.14 kg·ha^{-1} (Fig. 6*d*), ..."
(*ii*) On page 1561, 2nd column, under Other forage species, line 19 should read "... *thompsoni* (Figs. 6*f*, 6*g*, 6*h*)."
(*iii*) On page 1566, 1st column, line 24 should read "... df = 21,1092, P = 0.04; Figs. 6*b*, 6*e*) ..."

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